First detection and infestation levels of the invasive fruit fly *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae) in pomegranate orchards from Algeria

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Pomegranates are attacked by invasive fruit flies of the family Drosophilidae including the African fig fly, *Zaprionus indianus*. This species is reported for the first time from North-East Algeria reared from pomegranate fruits from two commercial pomegranate orchards. This vinegar fly is an opportunist that uses both fallen fruits and damaged fruits still on the pomegranate tree as breeding substrates. We report percent infestation of fruit of *Z. indianus* and of several other pomegranate insect pests.

Key words: *Punica granatum*, pests, vinegar fly, sap beetles, North Africa.

INTRODUCTION

The pomegranate (*Punica granatum* L.), and its human use is deeply embedded in history, utilised already by many ancient human cultures as food and as medicinal remedies (Holland *et al*. 2009). Pomegranates are a source of antioxidants, vitamins, potassium, calcium, magnesium, iron and zinc (Kahramanoglu & Usanmaz 2013). The antiquity of pomegranate can be attributed to dates of the Torah and the Bible. It is also mentioned in the Holy Koran (Kahramanoglu & Usanmaz 2016).

Different pests and diseases can cause both qualitative and quantitative damage to pomegranate fruit (Nobakht *et al*. 2015; Kahramanoglu & Usanmaz 2016). In Algeria, pomegranate cultivation is considered a minor crop, therefore little local information is available, especially concerning the pests that can affect production and fruit quality. The production of pomegranates is concentrated respectively in three regions: Djelfa (59 792 t), Msila (4572 t) and Naama (610 t) (DSA 2009).

Globalisation and climate change are causing the spreading of non-native insects, harmful to humans, agriculture and the environment (GEISCA 2013). *Zaprionus indianus* Gupta, 1970 is among the most widely documented invasive fruit pest species of the family Drosophilidae (Fartyal *et al*. 2014). The species, known as the African fig fly (Commar *et al*. 2012), is now recorded from several Mediterranean countries, and there is much concern because this species can infest fruits nearing maturity of many crops. It is considered a semi-cosmopolitan species (EPPO 2016). Aided by international trade and commerce, *Z. indianus* has been introduced to numerous countries outside of its native African range (Steffen *et al*. 2015; Willbrand *et al*. 2018). This fruit fly is now widely distributed in the Afrotropical, Nearctic and Palaearctic regions: Brazil (Vilela 1999; Pasini & Link 2012; Vilela & Goñi 2015), Uruguay (Goñi *et al*. 2001); Argentina (Lavagnino *et al*. 2008), and in Canada (Renkema *et al*. 2013). This fly has also successfully invaded India (Gupta 1970), Saudi Arabia (Amoudi *et al*. 1991) and Jordan (Al-Jboory & Katbeh-Bader 2012). In Europe, there is a questionable record from Spain, a male captured in Malaga (Carles-Tolrá 2009) and unconfirmed records from Italy and Austria (EPPO 2016). It has been recorded from the Canary Islands (Bächli 2013). In recent times, the introduction of *Z. indianus* in France was by importation of exotic fruits (Kremmer 2017).

There is conflicting information on the presence of *Z. indianus* in other EU countries (Steffen *et al*. 2015). Its presence has been reported in several countries surrounding the Mediterranean Basin. In northern Africa *Z. indianus* has been reported from many localities in Egypt (Yassin & Abou-Youssef 2004) and in Morocco (Yassin & David 2010). However, it has not been reported from any other North African countries. We report herein the first detection of *Z. indianus* reared from pomegranate fruits being grown in Algeria.

MATERIAL AND METHODS

Study area

The field work was undertaken in an agricul-

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tural zone near the salt lake, Chott Zahrez Echergui, a Ramsar Wetland, which is a flat basin formed of a sandy-clay substrate (Lemoalle & Morgan 1987). Insect pests were surveyed in two commercial pomegranate orchards (*P. granatum* var. 'hamodh') located in El Kharza (M'sila, NE Algeria; 3°39'48.80"E 35°13'46.27"N at 766 m altitude) and 17 km from the Sebkha of Chott Zahrez Echergui. Both orchards cover an area of 1 ha and consisted of a total of 300 pomegranate trees. These two adjacent orchards are distinguished by the age of their trees. Orchard 1 has 100 trees that are 47 years old and the Orchard 2 has 200 trees that are 89 years old. This area is semi-arid with cold winters. Precipitation is variable, with a mean of 207.5 mm and ranging between 105 and 348 mm per year. Annual mean temperatures are 19.43 °C (ranging between 8.73 °C to 31.63 °C). The dry period can last 11 months. Average annual evaporation estimated at 1050 mm per year. Both pomegranate orchards complied with all organic agricultural standards, *i.e.* no pesticides, fungicides or herbicides applied. Trees were regularly watered during dry periods by furrow irrigation. In association with the pomegranate trees were a community of diverse adventive plants including at least 16 species. The dominant species were *Marrubium vulgare* L. (Lamiaceae) and *Atriplex halimus* L. (Amaranthaceae). Other common species were *Sinapis arvensis* L. (Brassicaceae) and *Phalaris paradoxa* L. (Poaceae).

Insects were sampled on 24 October 2018 from both orchards. A total of 80 ripe fruits were removed from each orchard, 40 directly from trees and 40 fallen fruit from the ground. All fruit were intact and exhibited no noticeable blemishes or injury. Individual fruit from each orchard were kept in individual plastic containers covered with muslin cloth in the laboratory at ambient temperature (20 ± 1 °C) and relative humidity (40 ± 2 %). The containers were examined daily until no more adult flies were detected. The emerged adults and larvae were removed from containers, identified, and enumerated. All adults emerged between 24 October 2018 and 19 November 2018.

Identification and voucher specimens

Though the original objective of the survey was to identify fruit fly species (Drosophilidae) but other flies such as Tephritidae were identified along with other pests associated with the pomegranate fruits. Taxonomic determination of all insect pests has been confirmed by authors, M.K. and G.B. Fruit fly specimens were identified as *Z. indianus* using available keys (Tsacas & Chassagnard 1990; Chassagnard & Tsacas 1993; Van der Linde 2010; Yassin & David 2010). Voucher specimen of *Z. indianus* and all other insect pests are deposited in the Entomological Collection, Department of Agricultural Sciences, University Mohamed Boudiaf of M'sila, Algeria.

Data analysis

The parameters used to describe the pest community of the pomegranate fruits were species richness (*S*), relative abundance (*RA*) and percent infestation (*PI*).

$RA = (ni \times 100)/N$ (Triplet 2020)

where ni = individual number of pest species i and N = total individual number of all pest species.

For all samples, the percent infestation for each pest species (*PI*) were determined by the equation of Ouedraogo *et al*. (2010):

$PI =$ (Number of fruits attacked × 100)/Number of fruits collected.

The significant differences between the location of the sampled fruits (on trees or on ground) and impact on the pest infestations was estimated with non-parametric ANOVA (one-way). PAST software (PAleontological STatistics) Version 3.25 (Hammer *et al*. 2001) was used for statistical analyses.

RESULTS

Seven insect pests emerged from the pomegranate fruits sampled from both orchards. Three flies, *Z. indianus* and common fruit fly *Drosophila melanogaster* Meigen (Drosophilidae), and the well-known Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Tephritidae) were reared. In addition, two sap beetles (Nitidulidae), *Epuraea* sp., *Carpophilus hemipterus* (L.) (dried fruit beetle), and the pyralid moth *Ectomyelois ceratoniae* Zeller (carob moth) were reared. Throughout the study period no parasitoids were detected emerging from above insects.

Zaprionus indianus infested 37.5 % of total fruits collected from Orchard 1 and 20 % of the fruit by *D. melanogaster* (Table 1). Sixty percent of pomegranate fruit collected from Orchard 2 were infested by *Z. indianus* and 52.5 % by *D. melano-*

Table 1. Emerged adults of pomegranate pests in Orchard 1; relative abundance and percent infestation.

Pest species	Dropped fruits (*n* = 20)			Fruits from trees (*n* = 20)			Total (*n* = 40)		
	N	RA %	PI %	N	RA %	PI %	N	RA %	PI %
Drosophila melanogaster	84	48.84	30	9	18.75	10	93	42.27	20
Zaprionus indianus	57	33.14	40	22	45.83	35	79	35.91	37.5
Ceratitis capitata	0	0	0	2	4.17	5	2	0.91	2.5
Epuraea sp.	30	17.44	20	14	29.17	10	44	20.00	15
Carpophilus hemipterus	1	0.58	5	1	2.08	5	2	0.91	5
Total	172			48			220		

n: Number of pest individuals, RA: relative abundance, PI: percent infestation.

Table 2. Emerged adults of pomegranate pests in Orchard 2; relative abundance and percent infestation.

Pest species	Dropped fruits (*n* = 20)			Fruits from trees (*n* = 20)			Total (*n* = 40)		
	N	RA %	PI %	N	RA %	PI %	N	RA %	PI %
Drosophila melanogaster	85	20.99	55	178	54.10	50	263	35.83	52.5
Zaprionus indianus	200	49.38	70	114	34.65	50	314	42.78	60
Epuraea sp.	118	29.14	50	37	11.25	30	155	21.12	40
Ectomyelois ceratoniae	2	0.49	10	0	0	0	2	0.27	5
Total	405			329			734		

n: Number of pest individuals, RA: relative abundance, PI: percent infestation.

gaster (Table 2). From the infested pomegranate fruit, 393 *Z. indianus* and 356 *D. melanogaster* adults were reared. In both orchards, tree-picked fruits exhibited a high percent infestation by *Z. indianus* as compared to other pests (35 % *vs* 50 % for Orchards 1 and 2, respectively). Similarly, for fruits recovered from the ground from Orchard 1, *Z. indianus* comprised 40 %. However, Orchard 2 had a higher percent infestation (70 %) by *Z. indianus* (Fig. 1). There were no significant differences between location of the sampled fruits (on trees or on ground) and infestations ($P = 0.1764$ for Orchard 1; $P = 0.7748$ for Orchard 2) (Table 3).

DISCUSSION

Several pests and diseases are common to most of the pomegranate growing regions (Holland *et al*. 2009). *Zaprionus indianus* is not considered as a pest in its native area. This is not true for other regions where this fly is well established (Kremmer 2017). The African fig fly is included in the EPPO pest quarantine list in several countries (EPPO 2019). The detection of *Z. indianus* in temperate regions shows the capacity to adapt to conditions outside of its original tropical environment (Holle *et al*. 2018). It has been suggested, after establishment, long-distance migration could be involved, given the potential for long-distance movement observed with other fruit fly species like the spotted wing drosophila *D. suzukii* (Matsumura) (Asplen *et al*. 2015). This pest was a successful invasive species that presented high adaptive flexibility and extreme physiological tolerance (Mata *et al*. 2010). In our study, we report the coexistence of two fruit fly species, *Z. indianus* and *D. melanogaster* in the two pomegranate orchards. It is not known which species is the initial coloniser. In laboratory conditions, Vilela & Goñi (2015) has suggested that *D. melanogaster* can infest fig fruits after *Z. indianus*. This may be a case of ecological succession in the infestation process, in which *Z. indianus* is the pioneer, creating conditions in figs that are attractive to *D. melanogaster*, and fruit infestation succession may be mediated by different yeasts. The African fig fly with other drosophilids or fruit flies may actually increase damage to fruits (EPPO 2019). However, *Z. india-*

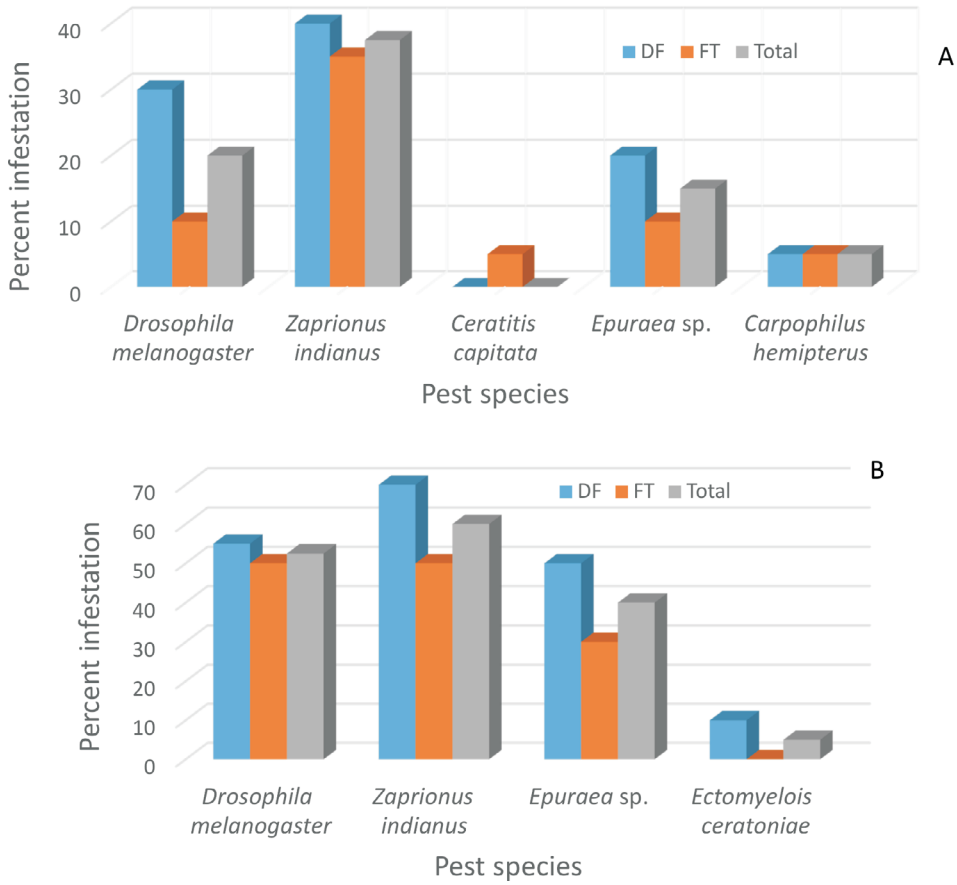

Fig. 1. Percent infestation of insect pests. **A**, Pomegranate orchard 1; **B**, pomegranate orchard 2.

nus is primarily considered as a generalist that breeds on fallen fruit and fruit on the tree. In Africa, it is known to infest fruits of more than 70 species from over 30 families of plants (Van der Linde *et al*. 2006; Lavagnino *et al*. 2008). As a polyphagous drosophilid, *Z. indianus* has been recorded from kiwi fruit, palm dates, grape, pomegranate, jujube, banana, loquat, and strawberry (Al-Jboory & Katbeh-Bader 2012; Renkema *et al*. 2013; Bernardi *et al*. 2017; EPPO 2019), and is able to attack over-ripened fruits on trees like oranges and peaches (Kremmer 2017). Larvae of this fly fed primarily on bacteria and yeast, particularly *Candida tropicalis* involved in fermentation substrates rich in carbohydrates of decomposing fruits (Gomes *et al*. 2003; Vilela & Goñi 2015). The volatile substances that originate in the process of fermentation of these substances function as the main attraction for these flies (Vilela & Goñi 2015). This fly has also been reared from wild fungi (*Phallus* sp.) in Brazil (Gottschalk *et al*. 2009). The drosophilid flies have been also implicated in the spread of the spores of fungal pathogens (Alford 2014).

Table 3. One-way ANOVA (position of fruits factor).

	Sum of squares	d.f.	Mean square	*F*	*P* (same)
Orchard 1	1537.6	1	1537.6	2.199	0.1764
Orchard 2	577.6	1	577.6	0.0876	0.7748

Zaprionus indianus has clearly demonstrated its invasive behaviour, and its ability to adapt to new environments and to use a wide range of fruit species (EPPO 2019). Furthermore, Pfeiffer *et al*. (2019) confirm that this invasive fly may be able to infest certain soft-fruit hosts. The capability to breed in immature fruits might give this species a dispersal advantage when compared to other

drosophilids that only breed on decomposing fruits (Mattos-Machado *et al*. 2005). The African fig fly has a wide range of fruit hosts and its ability to rapidly spread (Mattos-Machado *et al*. 2005) has contributed to its success in different regions. *Zaprionus indianus* and *D. suzukii* are the few drosophilid species considered to be primary pests (Walsh *et al*. 2011; Lasa & Tadeo 2015; Kremmer *et al*. 2017). In fig culture worldwide, the African fig fly is classified as a primary pest (Raga *et al*. 2003; Commar *et al*. 2012; Bernardi *et al*. 2017) only in the fig variety Roxo de Valinhos, but a secondary pest in other fig cultivars (Vilela & Goñi 2015). Possibly this fly is unable to act as a primary infester in other types of fruit in which the outer skin is intact (Steck 2005). As a secondary pest, adult *Z. indianus* females can oviposit only into fruits that have mechanical injury usually from other insects. This is related to the inability of females to oviposit on ripe fruit without prior injuries or the presence of mechanical damage caused by other insect pests (Fartyal *et al*. 2014; Bernardi *et al*. 2017). However, in our study all collected fruit exhibited no apparent physical injury. Additional studies are required to study oviposition behaviour on intact fruits.

Ceratitis capitata can occasionally cause serious damage to pomegranate. The extent of infestation depends on various factors, including susceptibility of the pomegranate variety, the seasonal weather patterns, and the presence of other suitable fruit crops in the cultivated area (Cocuzza *et al*. 2016).

Carpophilus hemipterus is often a serious pest of dried fruit but it can also provoke primary attack on mature fruit still hanging on trees, transmitting fungal and bacterial infections and is mainly associated with decaying fruits where larval development takes place (Mifsud & Audisio 2008). *Carpophilus* is the most important genus of sap beetles in regard to species that are of pest status (Williams *et al*. 1983). In date palm ecosystems, the layer of soft dates provides an environment that is conducive to beetle development (Warner *et al*. 1990). In Mediterranean areas, these sap beetles are polyphagous and common on fruit crops, attacking decaying fruits and sometimes ripe fruit on trees (Cocuzza *et al*. 2016). Recently, *Carpophilus* spp. are believed to have become a serious problem in Australian orchards, due to the decreasing use of broad-spectrum insecticides for the control of other stone fruit pests (James & Vogele 2000). Otherwise in the Middle East, growers try to enhance *Carpophilus* spp. as beneficial insects, using fermenting fruits and aggregation pheromones in Annona plantations, in order to improve fruit pollination (Emekci & Moore 2015).

CONCLUSION

Our survey of two pomegranate orchards in Algeria indicated that the African fig fly, *Z. indianus* is well established, infesting both fruits still on trees and fallen fruit. We suggested that routine monitoring and pest control should be adopted in Algeria to ensure a high quality of pomegranate production. We encourage integrated pest management programmes be developed to thoroughly understand the basic biology, ecology, and distribution of the African fig fly and other insect pests of commercial pomegranate production. The Institut National de Protection des Végétaux (INPV) and the European and Mediterranean Plant Protection Organization (EPPO) should be directly involved. Additional research, including more extensive surveys will be required to determine if *Z. indianus* consistently infests commercial pomegranate production throughout Algeria. The need to develop a local pest profile for pomegranate cultivation is imperative, emphasising phytosanitary programmes allowing international trade of pomegranate fruit.

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