goal, in future work, is to invest taxonomic efforts in parasitoid identification and/or descriptions, and to reveal the complex relationships among plant species (fruits), Drosophilids, and parasitoids.

Acknowledgments: We are grateful to the Universidade de Brasília and Reserva Ecológica do IBGE for logistical support, to Dr. Carolyn Elinore Barnes Proença and Msc. Marcelo Kuklmann for their valued help in plant identification, and to Msc. Anamaria Dal Molin for kindly providing us with bibliographic material for wasp identification. This work was funded with grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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Records of *Zaprionus indianus* and *Drosophila suzukii indicus* as invasive fruit pests from mid valley region of Garhwal Uttarakhand, India.

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Abstract

The present work is the first record of *Zaprionus indianus* Gupta and *Drosophila suzukii indicus* Parshad and Paika (Diptera: Drosophilidae) as invasive fruit pests from mid valley region of Garhwal, Uttarakhand. Different life stages of these flies were observed in Sweet orange (*Citrus sinensis* L.) and Guava (*Psedium guajava*). The female *Drosophila suzukii indicus*, widely known as spotted wing *Drosophila*, with

its serrated ovipositor lays eggs inside unwounded ripening fruits, while *Zaprionus indianus* females are unable to lay eggs through the skin of fruits and need injuries or wounds to lay their eggs. Here, we provide the description of the pests, their biology, life cycle along with extent of infestation which could be useful in devising future management plans and monitoring techniques.

Introduction

Zaprionus indianus Gupta 1970 and *Drosophila suzukii indicus* Parshad and Paika 1964 (Diptera: Drosophilidae) are among the most widely documented invasive fruit pest species of the family Drosophilidae. *Z. indianus* mostly attacks ripe and damaged fruit unlike *D. suzukii indicus*. The females of *Z. indianus* are incapable to insert eggs through the fruit surfaces; thus they mostly oviposit on the surface of previously damaged fruits (Tidon *et al*., 2003; Steck, 2005). *Z. indianus* adult females are also known to deposit eggs on the ostiole of fresh fruits like *Ficus carica* from where maggots penetrate the supple and fleshy internal core of the fruits (Vilela *et al*., 2001). They primarily feed on the yeast, like *Candida tropicalis* (Gomes *et al*., 2003) and bacteria found on decomposing fruits.

Drosophila suzukii and *Drosophila suzukii indicus* commonly known as spotted wing *Drosophila* is among those species that have been identified to oviposit in healthy, unwounded fruits in contrast to *Z. indianus*, which is mostly found on damaged or overripe fruits. The distinctive characteristics of *D. suzukii* make it an arduous pest are its proclivity towards fresh ripening (as opposed to overripe) fruit (Mitsui *et al*., 2006) and more importantly the presence of prominent serrated ovipositor of the female, which upon insertion causes physical damage to the fruit. Subsequently, these oviposition wounds caused by *D. suzukii* flies lead to secondary infections by insects and several other pathogens like bacteria, fungus, and yeasts, causing increased losses (De Camargo and Phaff, 1957; Molina *et al*., 1974; Louise *et al*., 1996). Also, the eggs laid often develop into larvae within the fruit, leading to fruit rot and overall reduced yields. Recently, Depra *et al*. (2014) gave the first record of *D. suzukii* from Brazil and raised the concerns towards its rapid dispersal throughout the region and potential menace to fruit culture.

Identification of Pests

Zaprionus indianus can be easily identified from other known species of this genus due to some distinguishing features (Figure 1a). It is somewhat yellowish, with even number of discrete silver-white stripes on head and thorax and 4 to 6 distinct composite spines on the anterior femora. These spines are not present on small tubercles and have a second short branch at its base which functions as a rest for the tibia of the folded leg. As compared to other species, the narrow black bands around the silvery thoracic stripes do not get wide on the scutellum, and the scutellum lacks a white tip (Van der Linde, 2006).

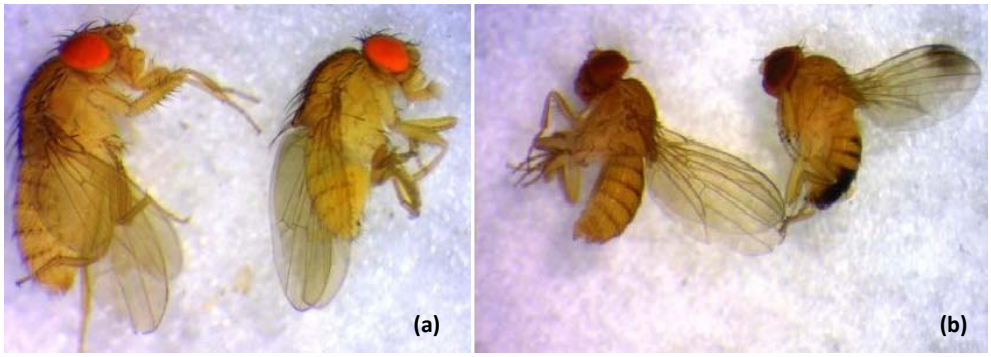

Figure 1. (a) *Zaprionus indianus* female and male fly, (b) *Drosophila suzukii indicus* female and male fly, respectively.

Adult *Drosophila suzukii indicus* are small (2-3 mm) flies with yellowish-brown thorax and abdomen and red eyes (Figure 1b). They also have black stripes on the abdomen and males have a distinguishing dark spot on the leading front near the tip of each wing. The females have a well serrated ovipositor, which aids in penetrating the most thin-skinned fruits leaving a small wound or depression on the surface.

Life Cycle

Zaprionus indianus females produce around 60-70 offspring on an average in their entire lifespan. The eggs laid are milky white in color and are typically in small masses laid by several females, mostly in damaged or fallen fruits with the exposed fruit pulp. The eggs are also laid in ostioles of some fruits while they are still on the tree, especially in figs (*Ficus* spp.). Transitions through the entire lifecycle (from egg to adult) may also be influenced by temperature. The average temperature of the study site during the sampling was around 18°-20°C, where the egg stage was for about 1-2 days, larval stage for 12-15 days, and pupal for 4-5 days. *D. suzukii indicus* on other hand had higher fecundity than *Zaprionus indianus*. Females on an average can lay up to a total of 400-500 eggs during their lifetime. These eggs hatch inside the fruits anywhere between 2-3 days, the larvae also matures within the fruit in about 5 to 15 days. Pupa mostly resides within the fruit for almost 4 to 12 days (Figure 2).

Figure 2. Life cycle of pests within infested fruits, (a) breeding, (b) emergence of larvae, and (c) pupae and larvae isolated from fruits.

This region experiences sub-tropical to temperate climatic conditions favoring sudden outbursts in abundance of this species. Similarly, population expansion after invasion of a few individuals favored by cooler climate more similar to the native range and areas previously invaded by this fly have also been reported from Brazil (Depra *et al*., 2014). Further, due to the short generation time and optimum temperature conditions in the region, these species achieve exponential growths within one season causing extensive damage to fruit crops.

Fruit Damage

Z. indianus was mostly found associated with damaged fruit on trees or felled off rotten fruits of sweet orange (*Citrus sinensis* L.). The larvae were even able to invade the soft tissue of over-ripened guava (*Psedium guajava*). On the other hand, *D. suzukii indicus* was able to lay eggs on healthy, unwounded fruit due to the serrated female ovipositor, preferring the ripening orange fruits over the over-ripened ones. Mostly the damage caused by *D. suzukii indicus* was due to its larvae feeding on fruit flesh. Moreover, the scar left behind due to insertion of the ovipositor into the skin of the fruit caused physical damage and called for secondary infections of pathogens fungi, yeasts, and bacteria leading to rapid deterioration and further losses (Figure 3). Another important observation was that the flies transferred and infested different fruits as seasons progressed and were not limited to one. The fact is currently looked upon in detail and will be communicated in further publications.

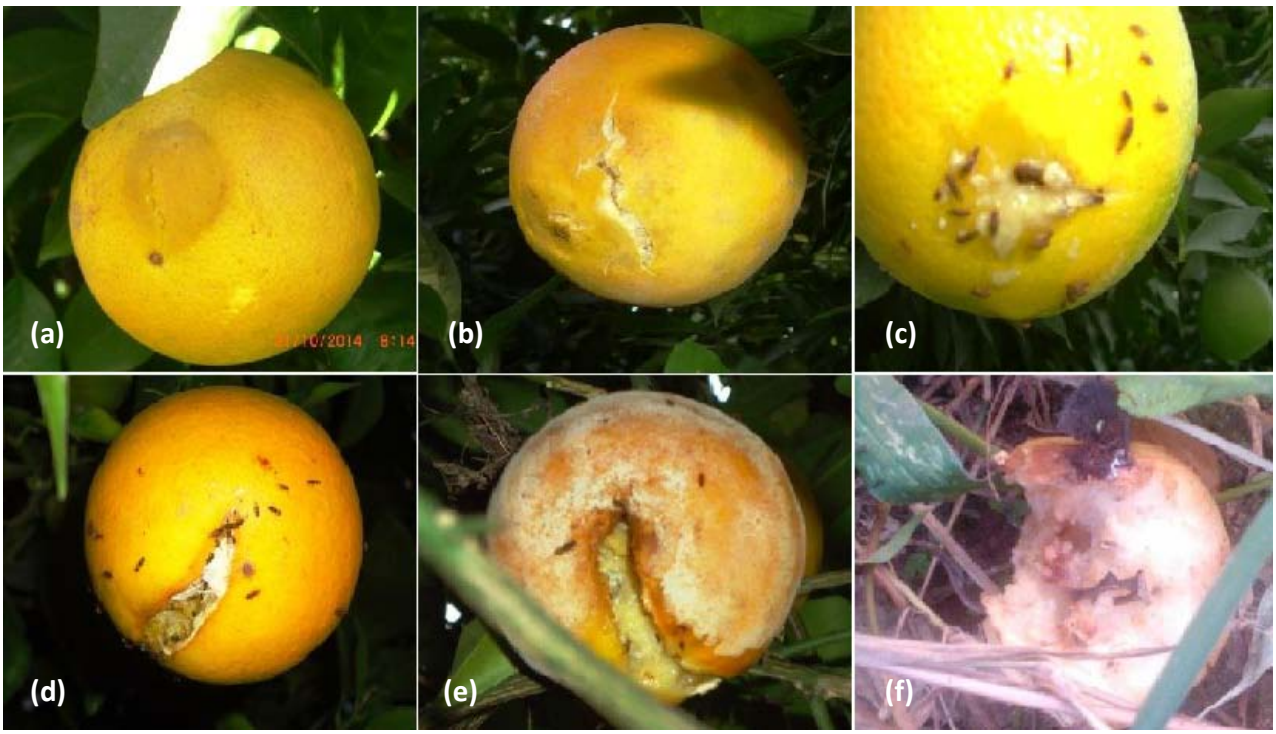

Figure 3. Different stages of fruit damage, (a) - (b) initial wound on sweet orange (*Citrus sinensis* L.), (c) - (d) infestation by pests, (e) secondary damage by fungal infection, and (f) *Z. indianus* larvae on fallen guava (*Psedium guajava*).

Eggs and larvae of the fly cannot be easily detected inside the fruits and some eggs and larvae can even survive periods of refrigeration (Kanzawa, 1939), which is often a menace to international fruit trade. This has been a plausible reason behind spread of *D. suzukii* to Europe and the USA (Rota-Stabelli *et al*., 2013) and recent invasion to Brazil (Depra *et al*., 2014). Thus, we also collected some fresh fruits from the vicinity of damaged ones. These fruits, along with some damaged by birds or other predators as well as the ones lying on the ground, were collected, brought to the laboratory, and were placed individually into sealed culture bottles (Figure 4) and kept at optimum temperature corresponding to the habitat. The bottles were examined every day for emergence of different life forms. We observed emergence of larvae even from some of those fruits that had no signs of physical damage. Subsequently, the adults that emerged were removed from bottles and identified. There were no records of emergence of the parasitoids throughout the culture period.

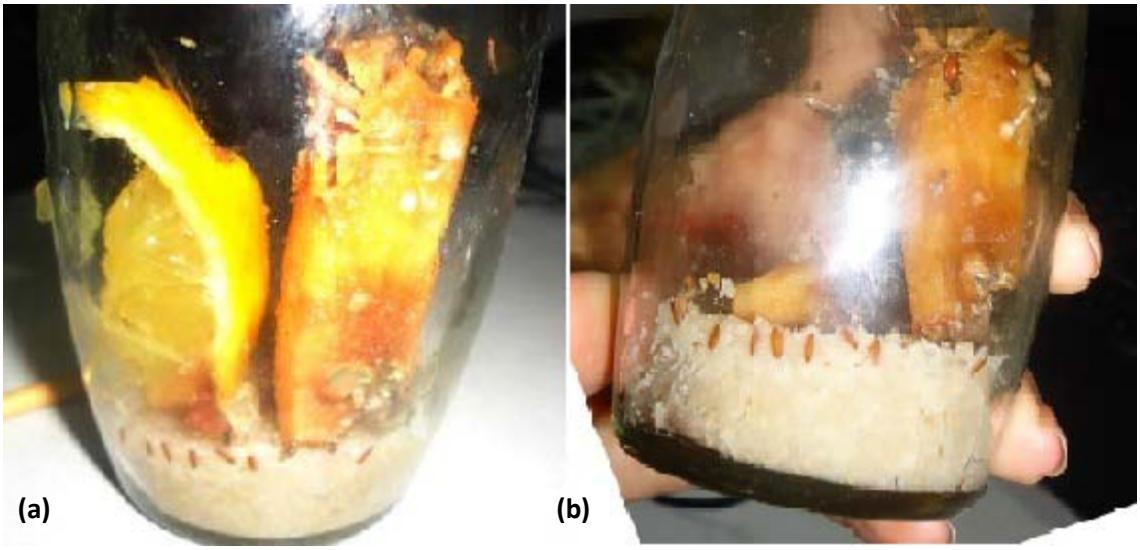

Figure 4. Laboratory culture of infested fruits depicting pupal and adult emergence, (a) sweet orange (*Citrus sinensis* L.), and (b) guava (*Psedium guajava*).

Conclusion

The present study provides the first records of *Zaprionus indianus* and *Drosophila suzukii indicus* as invasive fruit pests from mid valley region of Garhwal, Uttarakhand. The understanding of their basic biology, ecology, and distribution in this region would be helpful for development of efficient management strategies in future.

Acknowledgement: The authors deeply acknowledge financial support by University Grants Commission, New Delhi, India, as UGC-BSR fellowship to Manisha Sarswat and HNB Garhwal University fellowship to Asha.

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