Mangifera camptosperma Pierre, a wild relative of mango exhibits ovipositional antixenosis to Oriental fruit fly, *Bactrocera dorsalis* (Hendel)

Host plant resistance (HPR) refers to a particular defence trait of a plant that resists herbivore damage and can broadly be categorized into non-preference (antixenois), antibiosis and tolerance[1,2]. These host plant defence traits form the cornerstone of sustainable crop protection strategies across crops to battle insect damage. Therefore, understanding the underlying mechanisms of such host plant defences is crucial for researchers to exploit these plant traits for crop improvement[3]. Among different categories of HPR, non-preference refers to a physical (structural) or chemical trait of the host plant that makes it unsuitable for herbivore feeding or oviposition, thereby serving as the first line of defence[4]. In other words, herbivores totally avoid these plants over others due to certain plant traits[5]. Among plant structural traits (e.g. trichomes, spines, waxes, cuticles, etc.) that can act as physical barriers for herbivore feeding or oviposition, the plant cuticle and trichome density are the most important ones that are being exploited by researchers in the past to offset herbivore damage[3]. Similarly, chemical traits such as high levels of phosphorus, potash and polyphenols (against sorghum plant hopper, *Perigrinus maidis* (Ashmead)); procyanidin, *P*-hydroxybenzaldehyde, dhurrin (against green bug, *Scizaphis graminum* (Rondani)) and phenolics (against Oriental fruit fly, *Bactrocera dorsalis* (Hendel)) have been reported to play a role in oviposition antixenosis[6]. Nevertheless, in horticultural crops, HPR is being widely exploited in annuals rather than woody perennials.

Mango (*Mangifera indica* L., family Anacardiaceae) is widely being cultivated for its fruits. Among the herbivores that attack mango, *B. dorsalis* (Diptera: Tephritidae) cause significant economic loss (5–80%), as they attack ready-to-harvest fruits[7,8]. Female fly lays her eggs below the fruit rind and larval development takes place under the protective covering of the fruit rind. Few studies have evaluated the role of fruit pulp biochemical composition in relation to larval development (antibiosis) in selected polyembryonic (EC95862, Mylupilian) and monoembryonic (Langra) varieties of *M. indica*, emphasizing the defensive role of phenolics, and tannins against fruit fly larval development[6,8]. However, since fruits that are ready to harvest are attacked by their flies, the presence of single larva can result in fruit spoilage vis-à-vis market quality. Therefore, identification of potential antixenotic traits and their sources will help crop breeders incorporate these traits into commercial cultivars to offset fruit fly damage.

Wild species in general are considered as valuable sources of resistance to biotic and abiotic stresses[9]. Among ~60 wild *Mangifera* species that have been identified so far in Southeast Asia, many are becoming rare in their native habitats and appear on the IUCN Red List under various categories, viz. near threatened, extinct, etc.[10]. The present study was carried out on *Mangifera camptosperma*, a near-threatened species, native to Andaman and Nicobar (A&N) Islands, India[10,11], to get insights about the preference/non-preference of *B. dorsalis* to this species in comparison to commercial mango varieties.

Studies were carried out at the Division of Entomology and Nematology in collaboration with the Division of Fruit Crops, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru.

Green mature fruits of *M. camptosperma* were collected from the *ex situ* conservation blocks of the Institute (12°58′N; 77°35′E), where the scions brought from the A&N Islands were grafted onto *M. indica* root stocks and established successfully[10] (Figure 1). Green mature fruits of *M. indica* cv. Totapuri, a commercial cultivar known to be susceptible to *B. dorsalis* were used as control.

B. dorsalis were procured from the established colonies reared at the Fruit Entomology Laboratory, Division of Entomology and Nematology of the Institute. Insects were reared on banana as described earlier[12].

Headspace volatiles from green mature fruits of *M. camptosperma* and *M. indica* cv. Totapuri ($n = 5$) were collected using a customized air-entrainment system[13]. Volatiles were entrained from the fruits for 24 h and eluted with 800 µl of redistilled diethyl ether, and the samples were stored in a freezer (–20°C) until further use.

Fruits of both *M. camptosperma* and *M. indica* cv. Totapuri were exposed to *B. dorsalis* for egg-laying, where the gravid females (30 flies) were released into oviposition cage ($45 \times 45 \times 45$ cm) and allowed to acclimatize for 20 min. Both species of mango fruits ($n = 3$) were randomly placed together (choice assay) in the same cage and separately (no-choice assay) in different cages, and were exposed to fruit flies for 24 h; observations were recorded (the number of oviposition punctures/number of eggs laid for each fruit) under a stereomicroscope. The experiment was replicated five times. The data were subjected to a paired *t* test.

To understand the preference of gravid female *B. dorsalis* towards the headspace volatiles of *M. camptosperma* and *M. indica* cv. Totapuri, an assay was carried out using a Perspex four-arm olfactometer

Figure 1. ***a***, Plant of *Mangifera camptosperma* in the *ex situ* conservation blocks of ICAR-IIHR, Bengaluru. ***b***, ***c***, Flat and round fruits of *M. camptosperma*.

in a bioassay cage lit from the above by diffused, uniform lighting of fluorescent bulb (15W) and surrounded by black light-proof walls to prevent the influence of any external visual stimulus[13,14]. A total of 15 replications were carried out. Observations on the time spent in each olfactometer arm and the number of entries made into each arm were recorded using Olfa software (F. Nazzi, Udine, Italy). The data were compared by analysis of variance (ANOVA) and post hoc analysis with Bonferroni's multiple comparisons test.

Observations on fruit firmness (kg/cm^2), weight of peel, pulp and stone were recorded for both *M. camptosperma* and *M. indica* cv. Totapuri according to procedures earlier described[15]. The peel, pulp and stone weight were measured after hand peeling using a scalpel. The peel, pulp and stone were separated and weighed using an analytical balance. The data were analysed using *t* test. The total phenols and flavonoids from methonolic extracts of peel/pulp of both *M. camptosperma* and *M. indica* cv. *Totapuri* were determined according to earlier procedures[16,17].

Identifying potential genetic sources of HPR traits that resist herbivore damage is of great significance as it paves the way to incorporate these traits into elite cultivars either through conventional or advanced molecular crop breeding methods. Currently, the diversity of the elite cultivars has declined due to intense domestication and breeding for increased productivity[18]. Oriental fruit flies are the major problem in *M. indica* causing losses ranging from 30% to 80% depending on the variety[7,19]. Under these circumstances, screening wild *Mangifera* species may serve as valuable sources of HPR traits against fruit flies.

In choice assays, when both fruits of *M. camptosperma* and *M. indica* cv. Totapuri were exposed together, fruit flies did not lay eggs on the former fruits though gravid females alighted on them and attempted to insert their ovipositors. Significant difference in the number of ovipunctures ($P < 0.0001$, $t = 7.99$, d$f = 14$; *M. camptosperma*, 0.00; *M. indica* cv. Totapuri, 11.86 ± 1.49) and eggs ($P < 0.0001$, $t = 7.11$, d$f = 14$; *M. camptosperma*, 0.00; *M. indica* cv. Totapuri, 235.00 ± 33.07) per fruit was noticed. A similar trend was observed in the case of no-choice assay also, where flies did not lay any eggs on *M. camptosperma* fruits (number of ovipunctures per fruit: $P < 0.0001$, $t = 6.32$, d$f = 14$, *M. camptosperma*, 0.00; *M. indica* cv. Totapuri, 14.93 ± 2.36; number of eggs per fruit: $P < 0.0001$, $t = 6.91$, d$f = 14$, *M. camptosperma*, 0.00; *M. indica* cv. Totapuri, 321.67 ± 46.53).

The four-arm olfactometer bioassays revealed that gravid females spent significantly more time ($P = 0.05$; $F = 3.17$, e$df = 42$) in the regions treated with headspace volatiles of *M. camptosperma* (3.19 ± 0.92 min) and *M. indica* cv. Totapuri (2.89 ± 0.68 min) over control (0.98 ± 0.27 min). However, there was no significant difference ($P = 0.80$) in the time spent by gravid females between the regions treated with volatiles of *M. camptosperma* and *M. indica* cv. Totapuri. Similarly, the number of entries made by the gravid females into the treated regions was found to be significantly more ($P = 0.04$; $F = 3.41$, e$df = 42$) in *M. camptosperma* (2.73 ± 0.54) and *M. indica* cv. Totapuri (2.27 ± 0.37) compared to control (1.30 ± 0.21). Nevertheless, no significant difference ($P = 0.48$) was found between *M. camptosperma* and *M. indica* cv. Totapuri treated regions for the number of entries made by the gravid females.

The fruits of *M. camptosperma* are totally flat and round in shape with minimal non-edible fibrous pulp[20]. Observations on peel, pulp and stone of both *M. camptosperma* and *M. indica* cv. Totapuri revealed that there were significant differences in peel weight (*M. camptosperma*, 9.51 ± 1.26 g; *M indica* cv. Totapuri, 3.56 ± 0.32 g; $P = 0.0002$, $t = 4.54$, d$f = 13$), pulp weight (*M. camptosperma*, 10.05 ± 1.43 g; *M. indica* cv. Totapuri, 69.71 + 5.82 g; $P = <0.0001$, $t = 10.61$, d$f = 13$) and stone weight (*M. camptosperma*, 16.75 ± 1.76 g; *M. indica* cv. Totapuri, 21.67 ± 0.83 g; $P = 0.02$, $t = 2.28$, d$f = 13$). The phenols and flavonoids estimated from peel (*M. camptosperma*, 2.88 mg/g; 0.41 mg/g; *M. indica* cv. Totapuri, 4.23 mg/g, 0.35 mg/g respectively) and pulp (*M. camptosperma*, 1.25 mg/g, 0.26 mg/g; *M. indica* cv. Totapuri, 1.48 mg/g, 0.00 mg/g respectively) did not differ significantly among the species.

The fruit firmness of *M. camptosperma* was significantly more (66.15 ± 7.53 kg/cm^2; $P = 0.005$, $t = 3.13$; $n = 12$) compared to *M. indica* cv. Totapuri (42.11 ± 0.64 kg/cm^2). This would have contributed to the natural resistance of the fruit to flies (penetration resistance to ovipositor insertion by fruit flies). Earlier studies in the cherry fruit fly *Rhagoletis-cingulata* (Loew) also reported a positive association between reduction in fruit skin firmness and fruit fly infestation[21]. Similarly, *Rhagoletis pomonella* (Walsh) fruit flies did not infest black hawthorn fruits having penetration resistance >60 kg/cm^2 (refs 22, 23). Further, it was found that female fruit flies require ~10 sec to penetrate softer apples compared to harder fruits, where they need longer duration[24]. Thus, fruit firmness has been considered as an important trait that indicates host fruit susceptibility to fruit flies, as observed in other tephritid flies like *R. pomonella* and *R. cingulata*. In the present study also, *B. dorsalis* could not infest *M. camptosperma* fruits. This might be due to the fruit traits, viz. minimal fibrous pulp, more peel thickness and firmness. Here, the physical resistance of the fruit may hinder oviposition attempts by the fruit flies. Further, the excessively firm *M. camptosperma* fruit offers a biological barrier to female *B. dorsalis*, as flies must pierce through the skin of the fruit while laying eggs with minimum attrition to their ovipositors[23]. Therefore, it is clear that flies avoid fruits with thick peel in order to prevent damage to their ovipositors. Thus, *M. camptosperma*, a near-threatened species, native to A&N Islands exhibits ovipositional antixenosis to oriental fruit fly, *B. dorsalis*. Till date, there are no reports on sources of antixenosis to fruit flies, particularly in perennial crops like mango. This study indicates the potential of *M. camptosperma*, a wild relative of mango, that harbours crucial fruit fly oviposition deterrent traits which can be exploited through advanced breeding methods[25].

1. Painter, R. H., *Insect Resistance in Crop Plants*, MacMillan, New York, USA, 1951, p. 520.
2. Painter, R. H., *Annu. Rev. Entomol.*, 1958, **3**, 267–290.
3. Mitchell, C., Brennan, R. M., Graham, J., and Karley, A. J., *Front. Plant Sci.*, 2016, **7**; doi.org/10.3389/fpls.2016.01132.
4. Kogan, M. and Ortman, E. E., *Bull. Entomol. Soc. Am.*, 1978, **24**, 175–176.
5. Parsa, S., Sotelo, G. and Cardona, C., *J. Visual. Exp.*, 2011, **52**, 3047; doi:10.3791/3047.
6. Verghese, A., Soumya, C., Shivashankar, S., Manivannan, S. and Krishnamurthy, S., *Curr. Sci.*, 2012, **103**(5), 563–566; http://www.jstor.org/stable/24088755

7. Verghese, A. and Kamala Jayanthi, P. D., In *Pest Management in Horticultural Ecosystems* (eds Parvatha Reddy, P., Verghese, A. and Krishna Kumar, N. K.), Capital Publishing Company, New Delhi, 2001, pp. 1–23.
8. Kamala Jayanthi, P. D. and Verghese, A., *Pest Manage. Hort. Ecosystems*, 2008, **14**(1), 20–29.
9. Nass, L. L., Nishikawa, M. A. N., Favero, A. P. and Lopes, M. A., In *Recursos genéticos vegetais. Brasília, DF: Embrapa Recursos Genéticos e Biotecnologia* (ed. Nass, L. L.), ISBN: 978-85-87697-34-9, 2007, pp. 683–716.
10. Nischita, P., Dinesh, M. R., Ravishankar, K. V., Sandya, B. S. and Manoj Kumar, H. V., *Int. J. Curr. Res*., 2017, **9**(08), 55384–55388.
11. Ahlawat, S. P. S., *J. Andaman Sci. Assoc*., 2001, **17**, 1–2.
12. Kamala Jayanthi, P. D. and Verghese, A., *Curr. Sci.*, 2002, **82**, 266–268.
13. Kamala Jayanthi, P. D., Ravindra, M. A., Vivek, K., Roy, T. K. and Shivashankara, K. S., *PLoS ONE*, 2015, **10**(9), e0139124.
14. Kamala Jayanthi, P. D., Christine, M. W., John, C., Michael, A. B. and Toby, J. A. B., *J. Chem. Ecol*., 2012, **38**, 361–369.
15. Singleton, V. L. and Rossi, J. A., *Am. J. Enol. Viticult.*, 1965, **16**, 144–158.
16. Sudhakar Rao, D. V. and Shivashankara, K. S., *J. Food Sci. Technol.*, 2015, **52**, 4351–4359.
17. Chun, O. K., Kim, D. O., Moon, H. Y., Kang, H. G. and Lee, C. Y., *J. Agric. Food Chem.*, 2003, **51**, 7240–7245.
18. Trapero, C., Wilson, I. W., Stiller, W. N., and Wilson, L. J., *Front. Plant Sci*., 2016, **7**, 500; doi:10.3389/fpls.2016.00500.
19. Verghese, A., Madhura, H. S., Kamala Jayanthi, P. D. and John, M. S., In Proceedings of 6th International Fruit Fly Symposium, Stellenbosch, South Africa, 6–10 May 2002, pp. 317–324.
20. Dinesh, M. R. *et al.*, *Am. J. Plant Sci*., 2015, **6**, 2151–2160.
21. Teixeira, L. A. F., Rufus, I. and Larry, J. G., *Environ. Entomol.*, 2007, **36**(6), 1339–1348.
22. Messina, F. J. and Jones, V. P., *Ann. Entomol. Soc. Am.*, 1990, **83**, 742–752.
23. Mattsson, M., Hood, G. R., Feder, J. L. and Ruedas, L. A., *Ecol. Evol*., 2015, **5**(24), 5823–5837; doi:10.1002/ece3.1826.
24. Dean, R. W. and Chapman, P. J., *Agric. Entomol. Geneva*, 1973, **3**, 1–64; http://dx.doi.org/10.4236/ajps.2015.613217.
25. Migicovsky, Z. and Myles, S., *Front. Plant Sci.*, 2017, **8**, 460; doi:10.3389/fpls.2017.00460.

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A technique to collect male stingless bees

Stingless bees that belong to order Hymenoptera, family Apidae and tribe Meliponini, are one of the important beneficial insects acting as pollinators and honey-yielders[1–3]. There are about 500 described species belonging to 60 genera distributed in the tropical and subtropical regions of Africa, Australia, Indo-Malaya, Central and South America with the greatest diversity in the Amazonian rain forest of the New World[3–5]. In India, only 14 species of stingless bees belonging to three genera, namely *Lepidotrigona*, *Lisotrigona* and *Tetragonula* have been reported so far[5–7]. However, Rasmussen[5] while summarizing information on the described species of stingless bees from India predicts the potential of encountering several more species.

In stingless bees, males and females are extremely similar, making it difficult to distinguish sexes without microscopic examination[3,8–10]. However, male genitalia prove to be valuable diagnostic characters for distinguishing species complexes[5,9–11]. According to Rasmussen[5], as a first step to understand the full diversity of stingless bees of the Indian subcontinent, especially '*iridipennis*' species, taxonomic revision should include morphological characters of male genitalia, and it remains premature to describe and propose additional species of *Tetragonula* until their males are discovered. Attasopa *et al.*[11] also stressed the need to collect males and use them for the description of species. However, males are poorly represented in museum collections[10], as they are seasonal and produced occasionally[3,8]. In India, only three species (*Tetragonula iridipennis* Smith, *Tetragonula fuscobalteata* Cameron and *Lisotrigona chandrai* Viraktamath and Sajan Jose) are known by both males and females, while the rest only by females. Considerable effort and time are required to collect males of stingless bees[11], as swarming in Meliponini is infrequent[12].

Methods to collect males presently include continuous monitoring of nests for emergence of mating flights, searching for congregation sites (leks) of males[13], complete destruction of the entire nest, collecting foraging bees in specimen tubes and sexing them after killing or anesthetizing. However, the last method did not yield any male in an extensive survey conducted from 2008 to 2012 in seven states of India[14]. Attasopa *et al.*[11] emphasized that researchers make considerable efforts to collect males of Meliponini without destroying the nests.

During our efforts to collect males by monitoring colonies for the emergence of mating flights, we observed many foraging bees flying downwards immediately after coming out of the nest before moving away for foraging. In colonies nesting close to the ground (30–40 cm from the ground), foraging bees often fell down on their back, and then regained their position and flew away. This interesting behaviour lead us to explore the possibility of trapping such bees that may also include males, as male bees are known to leave the nest permanently after attaining maturity in search of virgin queens[3,8,12].