Abstract

The fig–fig wasp system is a brood site pollination system where pollinators breed inside an enclosed globular inflorescence (syconium) that they pollinate. This pollination mutualism is subjected to exploitation by the externally ovipositing non-pollinating fig wasps (NPFWs) that do not provide any pollination services but use the syconium flowers for the development of their offspring. Fig host location and oviposition by the mutualistic partner, i.e. the pollinating wasp, has been addressed adequately by fig researchers; however, host location by the exploiters has not been investigated. The main objective of this research work is to understand host location and host acceptance by externally ovipositing NPFWs in the cluster fig *Ficus racemosa*. The oviposition sites for these wasps, which are empty florets for gallers and galler larvae for parasitoids, are hidden within the fig syconium. With a community of six different species of NPFWs varying in their life history traits such as lifespan and ovigeny index and arriving at different times during syconium ontogeny to exploit the same resource for brood development, the syconium is converted into a microcosm. The syconium is a chemically diverse and crowded chamber that is dynamic in its occupancy over time and is accessed for egg-laying with the help of the probing ovipositor of the NPFWs that drill through the syconium wall from the exterior. Also, since fig wasp larvae are immobile and themselves do not have host-seeking behaviour, it is crucial for these parasitic wasps to accurately find the best oviposition sites for their offspring. We began our investigations with deciphering the trophic interactions within this multitrophic community, by investigating galler host–parasitoid pairings through manipulative experiments, and tracking the developmental trajectories of all NPFWs. The reproductive strategies of parasitoids provided exceptions to the conventional koinobiont–idiobiont dichotomy. The parasitoid *Apocrypta* sp 2 is a phytoentomophagous, ectoparasitic unconventional koinobiont and a relative generalist parasitising all three non-pollinating gallers *Sycophaga stratheni*, *Sycophaga testacea* and

Sycophaga fusca in their larval stages while *Apocrypta westwoodi* is an endoparasitic idiobiont and a specialist parasitoid parasitizing the pre-pupal stage of the early-arriving gallers *S. stratheni* and *S. testacea*. We succeeded in developing a novel lab set-up for raising fig wasps in lab from the pre-pupal stage onwards and observed that early-arriving gallers accelerate through their vulnerable stages probably to avoid predation by *A. westwoodi.* The possibility of a cryptic species within the population of *Apocrypta* sp 2 was suggested based on the large variation in its development time when arriving for oviposition at two different time periods during syconium development.

We then explored different factors that may affect the oviposition behaviour of these parasitic wasps as well as cues that may be utilised to gain information about potential oviposition resources. We demonstrated that the non-pollinating galler *S. fusca* distinguishes between syconia with varying oviposition history, prefers to oviposit into syconia into which conspecifics have oviposited, and that heterospecifics, in addition to conspecifics, can also affect oviposition choice in a system where multiple species use the same resource. We also observed that this oviposition preference is exhibited at the level of the syconium but does not extend to regions within a syconium that were exploited for oviposition. However, the preference for syconia into which conspecifics have oviposited may be lost when offered a syconium that has been over-exploited with regard to oviposition indicating a syconium oviposition threshold; this is possibly to avoid competition with conspecifics for egg-laying sites within the syconium microcosm. We also demonstrated that syconia with different oviposition histories differed in their volatile profiles, and the ones exposed to conspecifics had a characteristic chemical signature left by the footprints of ovipositing wasps. These chemical cues may reveal the identity of visitors to the syconium and thus indicate the suitability of the syconium as a resource patch for oviposition.

Since the NPFWs oviposit from outside the syconium, the ovipositor is the only organ that

comes in physical contact with hosts hidden inside the syconium. The ovipositor is therefore the major sensory organ involved in host location. We investigated the sensory nature of the ovipositor in NPFWs especially its olfactory nature. We recorded the electrophysiological response of the ovipositor to relevant stimuli using a modified electroantennogram (EAG) set-up. We call this novel set-up an ElectroOvipositogram (EOG).We amplified the signal-to-noise ratio of electrophysiological recordings from the fig wasp ovipositor by mounting the ovipositor such that its sensilla-rich tip was free to perceive volatiles. The ovipositor of the galler *S. fusca* showed an electrophysiological response to volatiles from pollen receptive-phase syconia to which that of the parasitoid *A. westwoodi* did not respond since this parasitoid does not seek galler host larvae at this phenological stage. The parasitoid's ovipositor, however, showed electrophysiological and behavioral responses to CO_2 in gaseous form possibly to seek out actively respiring galler larvae within their galls.We provide additional evidence for the porous nature of ovipositor sensilla in NPFWs by using techniques such as scanning electron microscopy, silver nitrate staining, and X-ray tomography. The ovipositor in gallers and parasitoids appeared to show adaptive responses to appropriate host and syconium stage volatiles.

Finally, we investigated the effect of stressful conditions such as ageing and host deprivation on host acceptance in NPFWs. The pro-ovigenic short-lived galler *S. fusca* showed a decrease in oviposition host specificity with age when host-deprived and, therefore, retaining a full egg load. This decrease in specificity takes place after the third day of adult life. However, specificity similar to that of freshly eclosed naive wasps is maintained if aged wasps are allowed to oviposit, suggesting that egg load interacts with biological age to affect oviposition behaviour. The oviposition host- and syconium stage-specificity of synovigenic and longer-lived parasitoids remained unchanged with age within the experimental limit of this work suggesting that oviposition in the wrong host may result in zero reproductive fitness

and that there is a premium on high specificity at all ages. Interestingly, another pro-ovigenic and short-lived galler *S. testacea* did not accept the non-host stage syconia that were offered for oviposition despite being time-limited as a result of being aged. This suggested the possibility of a morphological limitation, i.e. the length of ovipositor was too short to reach oviposition sites, and therefore precluded the demonstration of behavioral plasticity in this species. We, therefore, demonstrate how stressful conditions such as ageing or oviposition deprivation may differently affect species that vary in life history traits.

This thesis has addressed several challenging questions. We investigated the trophic interactions of an entire fig wasp community, and we also succeeded in raising fig wasps outside the syconium for the first time. Oviposition behavior in parasitic fig wasps was investigated at the level of individual wasps with a parallel focus on changes induced by physiological stressors such as ageing. We were also successful in demonstrating the olfactory nature of the fig wasp ovipositor especially its response to carbon dioxide in gaseous form and have highlighted the role of the ovipositor in exploiting hidden oviposition resources. The results of this thesis contribute to furthering of our present understanding of the existence of exploiters in the 75+ million year old co-evolved nursery pollination mutualism of the figs and their pollinator wasps. This thesis has also opened up many fruitful areas for novel research.