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Fig volatiles: their role in attracting pollinators and maintaining pollinator specificity

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Abstract: Each fig tree species (*Ficus*) is totally dependent on a specific species of wasp for pollination and the larvae of these wasps only develop in the ovules of their specific *Ficus* host. Because the fig crop on any particular tree is generally highly synchronized, the short-lived female wasps must leave their natal tree in order to find figs which are suitable for oviposition. Chemical volatiles produced by figs when they are ready for pollination are thought to be the means by which the wasps detect a suitable host. Gas chromatograms of the fig volatiles of 7 species of *Ficus* showed them to be species specific. Age related changes in the volatile profiles were noted as extra volatiles are produced when the figs were ready for pollination.

The relationship between fig trees (*Ficus* spp., *Moraceae*) and their pollinating wasps (*Chalcidoidea, Agaonidae, Agaoninae*; sensu BOUČEK 1988) is often considered to be the extreme example of plant-animal coevolution (JANZEN 1979). There are some 750 *Ficus* species worldwide (BERG 1988), each of which is generally pollinated by females of its own specific species of wasp (WIEBES 1979, MICHALOUD & al. 1985, WIEBES & COMPTON 1990). The trees are totally dependent on the wasps for pollination, while the wasp larvae develop only in the ovules of their *Ficus* hosts.

Figs (also called syconia) are hollow, roughly spherical inflorescences, lined on their inner surface with hundreds of unisexual flowers. The pollinating female wasps enter the fig through the bract-lined entrance (the ostiole) and pollinate the flowers, some of which are also used for oviposition. These foundress wasps usually lose their wings during the passage through the ostiole and are unable to leave.

Fig development can be divided into five distinct phases (GALIL & EISIKOWITCH 1968). In the prefemale phase, the ostioles of the young developing figs have not yet opened. This stage is followed by the female phase where the female flowers mature and the ostiole opens to allow the pollinators to enter the fig. Once pollination has taken place the figs enter the inter-floral phase where both seeds and wasp larvae are developing. The male phase commences with the maturing of the male flowers and the emergence of the wingless males of the pollinator wasp which seek out and mate with the female wasps while they are still in their natal galls. After emerging from their galls, the pollinator females acquire a load of pollen either actively or passively (GALIL & EISIKOWITCH 1973). They then leave their natal fig through a hole chewed through the fig wall by the males. Finally, the fig ripens (post-floral phase) and attracts various avian or mammalian frugivores which disperse the seeds (JANZEN 1979).

Fig development on individual trees is normally highly synchronized, forcing the short-lived adult wasp females (KJELLBERG & al. 1988) to leave their natal trees and search elsewhere for figs containing flowers that are ready to be pollinated. Factors involved in host finding and host specificity are only partially understood. A potential attractant is chemicals released from the figs (JANZEN 1979, RAMIREZ 1970). BRONSTEIN (1987) provided indirect evidence for chemical attraction when she showed that large numbers of pollinators of the neotropical *F. pertusa* L. arrived at their host tree only when the figs were ready to be pollinated. Confirmation of long distance chemical attraction was provided by VAN NOORT & al. (1989) who showed that the pollinators of *F. burtt-davyi* HUTCH. were attracted only to the figs of their host *Ficus* and this only occurred when the figs were at the appropriate stage of development.

There have been few previous studies of the volatiles released by fig trees. JENNINGS (1977) found that the differences between the steam distillate volatiles of ripe figs from 4 cultivars (some are gynocarpic and do not require the services of the pollinating wasps to set fruit) of *F. carica* L. were only quantitative. Other studies have concentrated on either the leaf volatiles (BUTTERY & al. 1986) or the composition of volatiles from stem exudates (WARTHEN & MCINNES 1989), but neither leaves nor stems play a role in attracting fig wasps (VAN NOORT & al. 1989). BARKER (1985) provided gas chromatograph evidence of the existence of fig volatiles.

Host specificity of *Ficus* species is likely to be achieved through a combination of these long distance volatile attractants, short range, contact stimuli provided by the fig surface and other physical characteristics of the fig. These may include the chemical properties of the fig surface (WARE & COMPTON 1992) and the physical characteristics of the fig ostiole (RAMIREZ 1974, JANZEN 1979) through which the wasps must crawl in order to reach the fig flowers and oviposit (GALIL 1977).

In the present paper, we address questions related to the chemical nature of the long distance attractants produced by figs. Initially, we determined whether the figs of each *Ficus* species has a characteristic bouquet, a possible means by which the wasps could distinguish their host free species from other *Ficus*. Changes in the composition of the bouquet of the figs of several species were then examined in relation to their developmental cycle. Changes observed in the volatile profile of the figs during the period when the fig flowers are ready for pollination could account for the observation that wasps are attracted only to the trees at this stage of fig development.

Material and methods

The volatiles of seven *Ficus* species were investigated: *F. sur* FORSSK., *F. burtt-davyi, F. thonningii* BL., *F. lutea* VAHL, *F. ingens* (MIQ.) MIQ., *F. macrophylla* DESF. from the Grahamstown area, eastern Cape Province, South Africa, and three cultivars of *F. carica*

(Calimyrna, Kardota, and White Genoa) from the Citrusdal area of the western Cape Province, South Africa. *F. macrophylla* is native to Australia while *F. carica* is of Mediterranean origin. The other species are native to South Africa.

Cotton bags were used to enclose prefemale stage figs in order to prevent wasps from pollinating the figs. Once the figs had reached the attractive female phase, determined by confirming that wasps had entered other figs on the same tree, they were harvested and within 10 min were placed in a glass tube (internal diameter 30 mm, length 300 mm). Air cleaned with activated charcoal was directed over the figs at approximately 1 l/min for 5 h and the volatiles, chemicals in the vapour phase, that were released trapped on activated charcoal (Orbo 32, Supelco, Bellefonte, PA). The volatiles of unpollinated prefemale and pollinated inter-floral stage figs were processed in a similar way. With the exception of the locally scarce *F. lutea* and *F. macrophylla*, prefemale, female, and inter-floral stage figs from at least three trees of each species were analyzed independently. The number of figs processed depended on their size. For large figs such as *F. carica* as few as 8 figs were used, while for *F. burtt-davyi*, the species with the smallest figs, at least 20 figs were used during each volatile trapping experiment.

Volatiles were eluted from the charcoal traps with 1 ml dichloromethane (Merck Cat No 6048). The eluant was then sealed in glass ampoules and stored at 4 °C. When required, the contents of each ampoule were concentrated to approximately 10 µl by evaporation with a stream of nitrogen, and 1 µl of the resultant concentrate was chromatographed on a fused silica capillary column (SGE; 25 m with an internal diameter of 0.22 mm) on a Hewlett Packard (HP) 5890 gas chromatograph (GC) fitted with a flame ionization detector and using nitrogen as a carrier gas. The instrumental parameters were: injection port temperature 210 °C, flame ionization detector temperature 210 °C, nitrogen carrier gas 20 ml/min. The initial oven temperature of 40 °C was maintained for 1 min and then was increased at a gradient of 5 °C/min to a maximum temperature of 180 °C, which was then maintained for 5 min. The temperature was then increased at a rate of 10 °C/min until the oven temperature reached 250 °C which was maintained for 10 min before the run was terminated. Purge time for the injection port was set at 0.5 min. The results were analyzed on an HP 3393A integrator, the attenuation being set to zero.

Results

Differences in volatile profiles. The volatiles released from the female phase figs of the seven *Ficus* species each resulted in a unique gas chromatogram (Figs. 1, 6). All the chromatograms were complex, containing many peaks each of which represented an individual volatile compound. Most of the volatiles were present in trace quantities (a full scale deflection at an attenuation of zero represented approximately 5 ng of material), some of which may be caused through the degradation of the figs, insect damage or even directly from small insects such as scale insects. The profiles from different individual trees of the same species were generally similar (see below for an exception) showing that each tree species has its own characteristic bouquet. For example, the volatile profiles of the three cultivars of *F. carica* were found to be essentially similar, differing quantitatively rather than qualitatively (Fig. 2). The general uniformity within species was observed in the prefemale phase chromatograms of some ten *F. burtt-davyi* (Fig. 3). However, the female phase figs of a further 2 trees were found to contain an additional major peak, which eluted at ca. 12 min.

Age related changes in volatile profiles. The chromatograms of *F. burtt-davyi* figs at the female stage of development showed an additional volatile eluting at ca. 12 min (Figs. 3, 4). As mentioned above, some trees produced a further additional

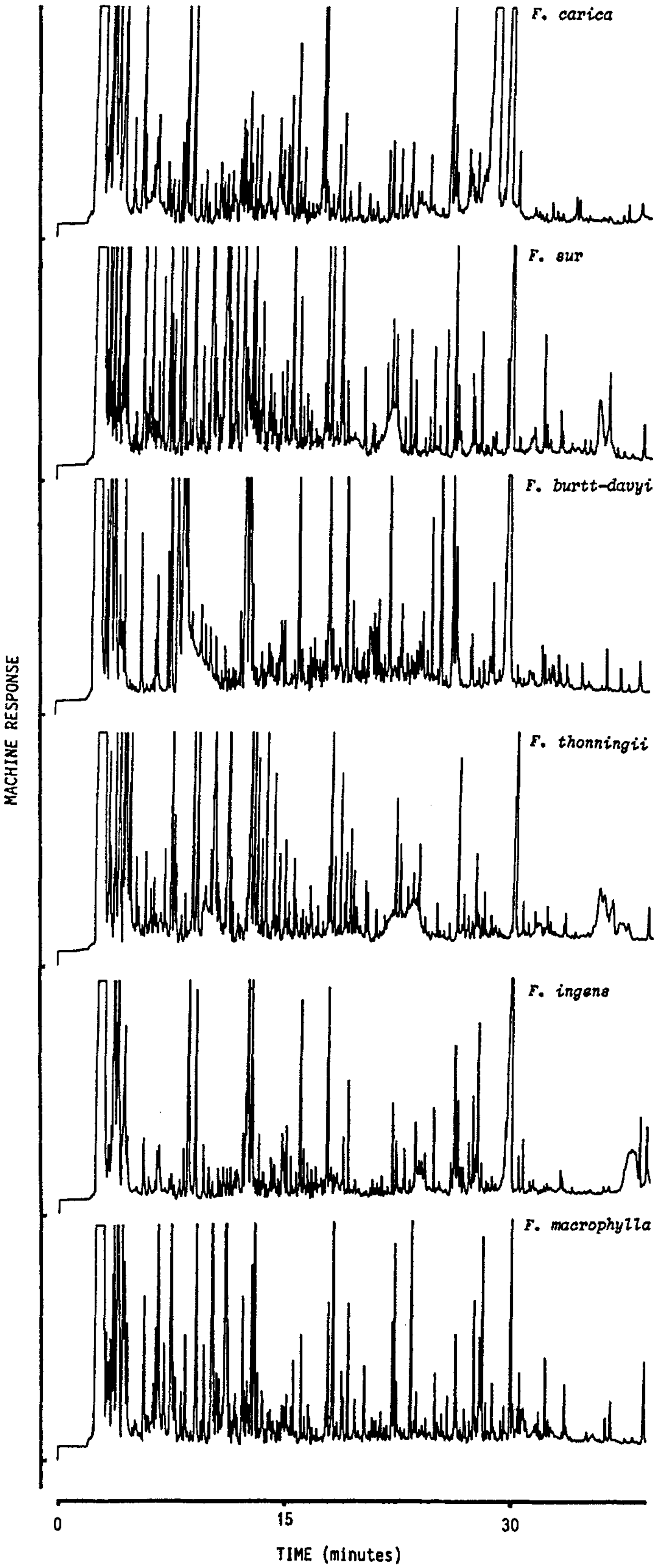

Fig. 1. Chromatograms of volatiles from female phase figs from six individual trees of six *Ficus* species. A full amplitude response at the detector represents at least 5 ng of material while the retention time indicates how long the volatiles remained on the column before reaching the detector. The smaller more volatile compounds generally elute first while the oven temperature is still low. See text for instrumental parameters

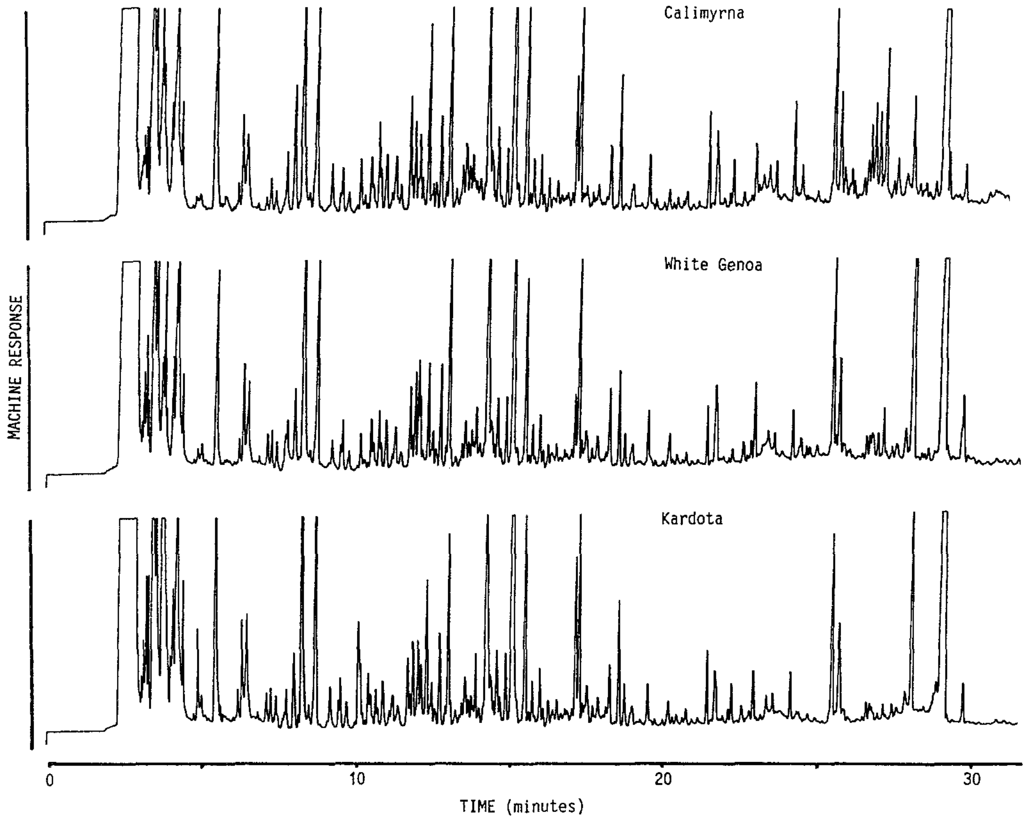

Fig. 2. The volatile profiles of female phase figs from three cultivars of *Ficus carica*

peak with a slightly reduced retention time at this stage of their development (Fig. 3). The volatile profiles of prefemale and inter-floral phase figs of *F. burtt-davyi* were similar (Fig. 4). Similarly, in *F. ingens* the female phase figs produced extra volatiles that were not recorded before or after this stage of development. In this case there were consistently two additional peaks, with retention times of ca. 24 and 25 min (Fig. 5). One additional peak was present in the female phase chromatogram of *F. lutea* with a retention time of ca. 12 min (Fig. 6). Unfortunately, no inter-floral fruit was available for comparison because most of the figs of *F. lutea* figs were not pollinated.

Discussion

Flower volatiles play a vital role as olfactory cues in attracting pollinating insects (Pellmyr & Thien 1986). Figs are no exception in attracting their pollinators, even though their flowers are contained within a syconium. Many insects use these olfactory cues together with visual stimuli such as colour (Tabashnik 1985, Owens & Prokopy 1986) and shape (Rausher 1978, MacKay & Jones 1989) to find their host plant. Van Noort & al. (1989) have shown that the wasp pollinators of *F. burtt-davyi* do not require these additional visual aids to find receptive figs of their host.

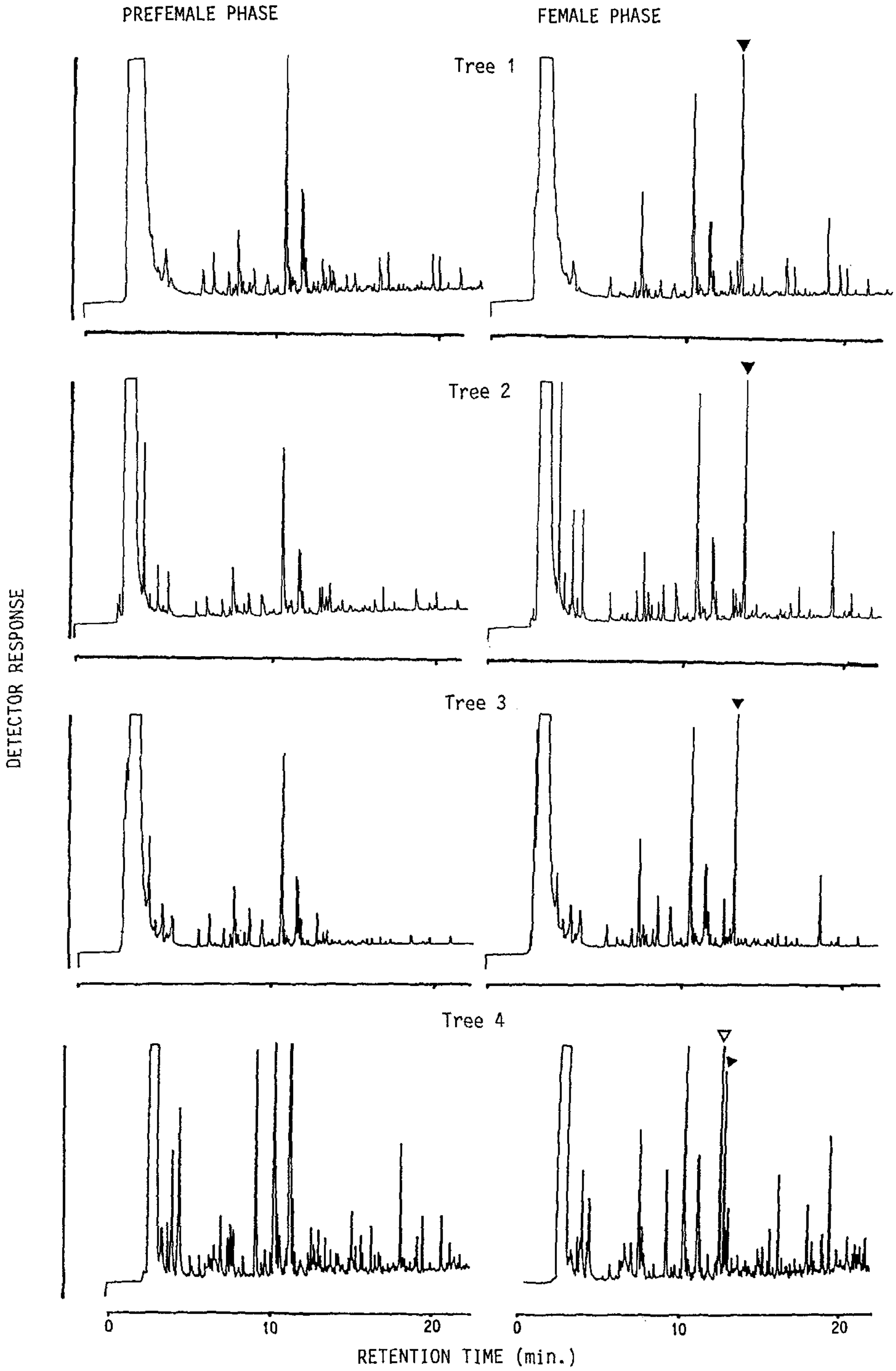

Fig. 3. The volatile profiles of prefemale and female phase figs of four individual trees of *Ficus burtt-davyi*. The closed symbol highlights the additional volatile recorded from figs in the female phase. The open symbol indicates that volatile which was released from female phase figs of two individual trees

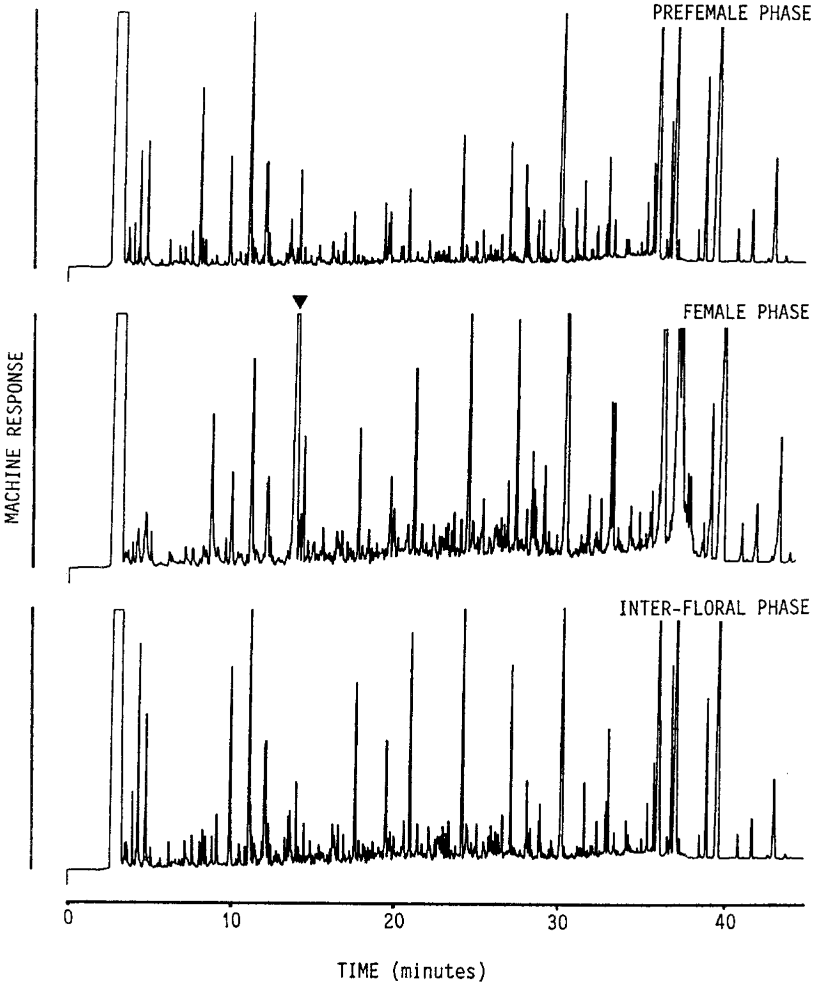

Fig. 4. The gas chromatograms of prefemale, female, and inter-floral phase figs from *Ficus burtt-davyi.* The closed symbol indicates the additional volatile peak occurring in the volatile profile of female phase figs

The movement of volatile molecules in the atmosphere is complex (Murlis & al. 1992). To be effective and reliable sources of information, volatile attractants have to be consistently emitted and easily distinguished from the background of naturally occurring odours. Electrophysiological studies have shown that cues resulting from a single volatile compound are probably the exception rather than the rule (Visser 1986). This implies that the fig volatiles are probably an uncommon mixture of compounds of the immediate environment and present themselves in reasonable amounts only when the figs are ready to be pollinated.

In pollinating systems such as those between some orchids (*Ophrys*) and male bees, the partnership can also be highly specific (Hills & al. 1972, Borg-Karlson & al. 1985). Here, each orchid species possesses a unique blend of volatiles, components of which may mimic the pheromones of attractive female bees. The plants deceive the male bees which, while attempting to copulate with them, pollinate the

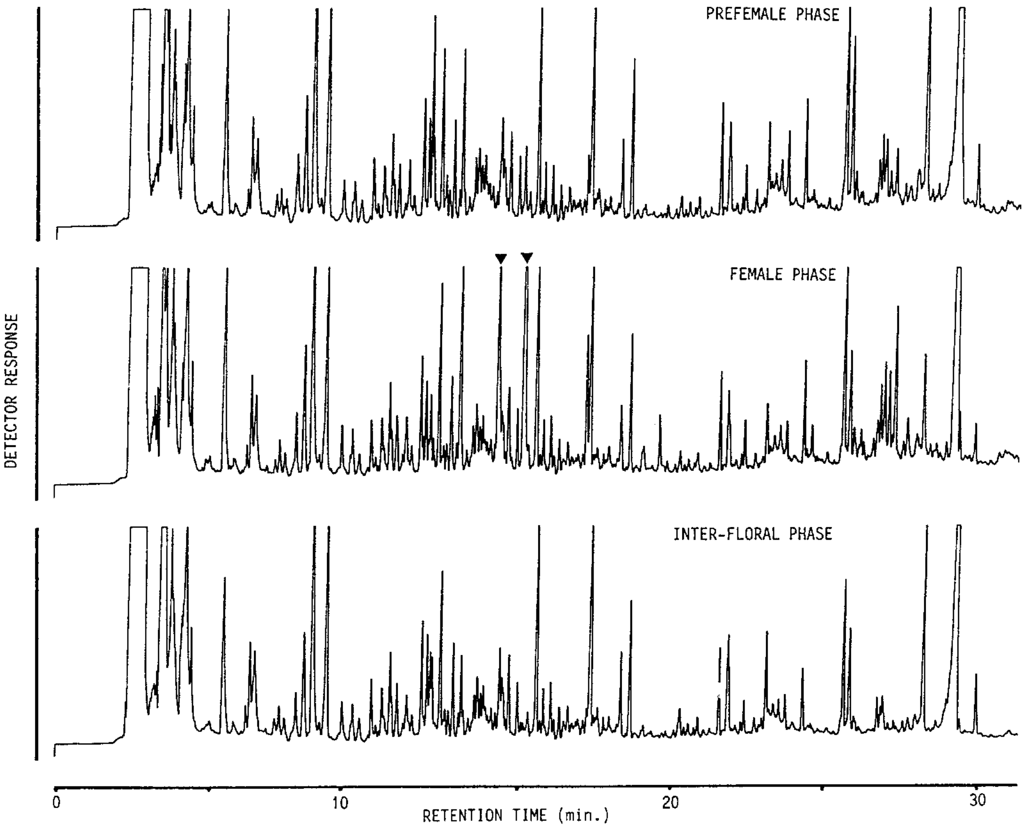

Fig. 5. Gas chromatograms of the volatiles from prefemale, female, and inter-floral stage figs of *Ficus ingens*. The symbols indicate additional volatile components produced by female phase figs

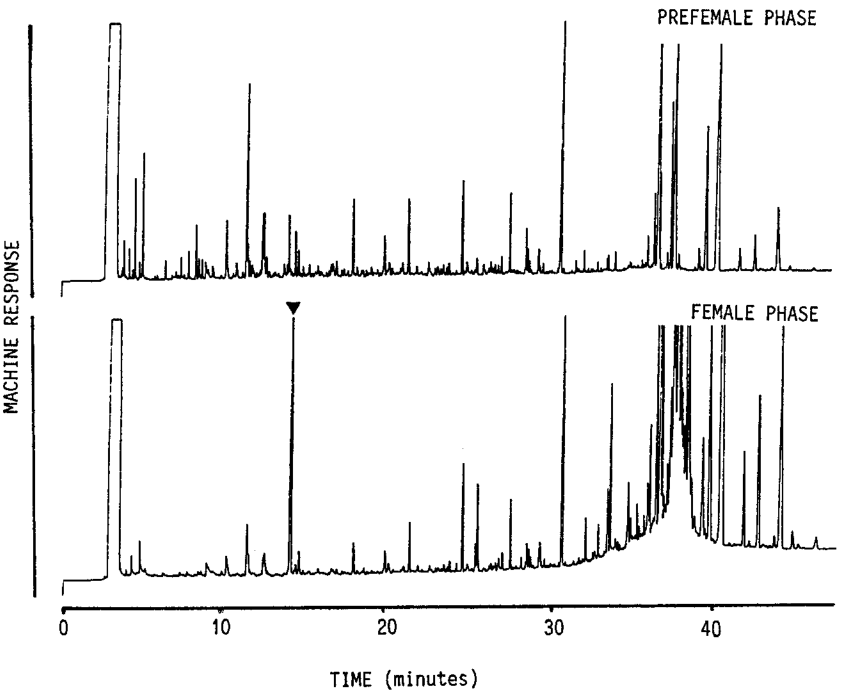

Fig. 6. The volatile profiles of the prefemale and female stage figs of *Ficus lutea*. The symbol indicates the additional volatile produced by female phase figs

flowers. Among such orchids, speciation potentially results from mutations which lead to changes in the plants' attractive volatiles (HILLS & al. 1972).

Similarly, the volatiles produced by figs may facilitate the obligate relationship between fig trees and their pollinators. The figs of each *Ficus* species produce a different bouquet of volatiles, which is largely consistent within species, allowing host specific pollinators to differentiate between them. Furthermore, additional volatile(s) are released at the time the pollinators are attracted. Presumably it is these additional compounds, either alone or in combination with the "normal" volatile bouquet, which form the basis of attraction. Female phase volatiles could, therefore, be of biological significance because this is the period when pollinators are attracted to their respective host trees. Identification, synthesis, and bioassay of the compounds are now required in order to confirm these findings.

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