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Evidence of a volatile attractant in *Ficus ingens* (Moraceae)*

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ABSTRACT

The relationship between the fig *Ficus ingens* (Miq.) Miq. and its wasp pollinator, *Platyscapa soraria* Wiebes, was studied. A mechanism of pollinator attraction is discussed and corroboration obtained by using gas chromatography to show the presence of volatile compounds. These compounds are shown to be released at the female phase of the fig's flowering cycle. These data are correlated to pollinator behaviour and visitation data obtained from field observations.

INTRODUCTION

The close relationship between the fig and its wasp pollinator has long been known, and there are numerous studies that have shed much light on the complexity of this relationship, such as Ramirez (1970, 1974) Galil & Eisikowitch (1974), Galil (1977), Janzen (1979a) and Wiebes (1979), to mention but a few of the more modern studies.

In papers that concern pollinator behaviour several authors such as Hill (1967), Galil (1977) and Janzen (1979a) have stated that the method of pollinator attraction is olfactory, but this is assumed and no evidence is provided. Janzen (*l.c.*) states in his conclusion, as well as elsewhere in his paper, that 'This pollination is achieved at a very low density of flowering trees, probably by chemical attraction of the wasps'. Ramirez (1970) cites several examples of long-distance pollinator attraction in the genus *Ficus*.

The complex interaction of the life cycles of the fig and the wasp will not be discussed here in detail, as this aspect has been well researched by workers such as Ramirez (1970, 1974), Galil & Eisikowitch (1968a, 1968b), Galil (1977), Wiebes (1979) and Janzen (1979a).

In this study an attempt was made to discover whether or not *Ficus ingens* produces an odour or scent, and to determine if this scent production coincided with the receptive female phase of the fig tree.

MATERIALS AND METHODS

Study sites

Plants of *Ficus ingens* were studied at a site on the Braamfontein Spruit, in the suburb of Craighall Park, Johannesburg. The site consisted of a population of 23 fig trees of varying sizes (three seedlings, 12 established trees and the rest large, mature trees) growing on the northfacing aspects of granite outcrops. Throughout the nine months of observation, it was noted that, at any one time, no more than one tree was at a particular flowering stage. There was, however, one tree at the male phase (wasp-hatching phase) and one tree at the female phase (egg-laying stage for the wasps) during August. This meant that the wasps were emerging from syconia of one tree and flying to the next, a few metres away. During the study period evidence for flowering was found on only seven trees in the population. Most of these seemed to have just finished flowering. This is a little unusual, as more of the population should have been in flower. The serious drought and winter weather may have had an effect on the flowering of the trees. The site was visited every two or three days, and every day for the duration of peak syconial activity.

Herbarium specimens of the studied trees are housed in the Moss Herbarium, University of the Witwatersrand (J). Specimens of the wasps are housed in the Zoology Museum, University of the Witwatersrand.

Scanning Electron Microscopy

Wasps emerging from syconia were captured in a trap consisting of a wire frame and nylon stocking, which was tied over a branch with syconia of the correct age. This age was determined by the increased size and slight colour change in the fruit. Newly hatched wasps could be found inside the syconium when it was split open, indicating the correct syconial state.

When a sufficient number of the wasps had emerged, the twig was cut off, and the trap then taken (with twig *in situ*) to the laboratory. The stocking was then cut away from the wire and twig, and the wasps collected and anaesthetized. These wasps were then stored in 100% alcohol and kept for viewing. They were then critical point dried, placed on a SEM stub, coated with gold-palladium and studied. Photographs were taken when required.

Gas chromatography

Syconia of the female phase were collected and taken to the laboratory on ice. They were then divided up into size classes, ranging from 6 mm and less to 9 mm and more, at intervals of 1 mm as measured across the diameter.

These were then placed in small glass bottles with glass stoppers. The bottles had been cleaned sequentially with distilled water, acetone and chromic acid. The atmosphere inside the bottles was then allowed

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to equilibrate for 30 minutes, the bottles being kept at room temperature.

The control consisted of an empty bottle that had been subjected to the same cleaning treatment. The atmosphere inside this bottle was analysed in the same manner as the samples.

The method used in the chromatographic analysis was the solvent effect, as described in Grob (1975). This method involves the shut-down of the carrier gas supply to gas chromatograph (GC), and the injection of 25 μl of a suitable solvent (in this case n-Hexane). This is injected by a glass and stainless steel syringe. Fifteen seconds after this first injection, 3 ml of the atmosphere from the bottles is injected using a clean gas-tight syringe. One minute after this second injection, the carrier gas flow is resumed and the temperature program initiated. The advantages and theory of this method are outlined in Grob (1975).

The temperature program that was run started at a temperature of 40°C (held for 1 minute) then increasing at a rate of 1,3° per minute. This program was run for approximately 30 minutes per run. The output of the GC was integrated by a Hewlett Packard HP3390A integrator, using the following parameters: attenuation of 0 and an area of rejection of 10 000. The paper speed was set at 0,5 cm per minute. The column used in the GC was a 25 m-long methyl silicone filament column, and the GC was a Carla Erba Strumentazione Gas Chromatograph.

After the syconia had been used in the bottles, they were removed, opened longitudinally and the number of wasps inside noted for each size class.

Fifty syconia of *Ficus ingens* were examined to see if a relationship between the presence of the exudate and the presence of wasps existed. This was done by collecting syconia with and without this exudate (from one tree) and noting the presence or absence of wasps inside the syconia. The results obtained were statistically analysed using a two by two Chi-squared contingency test with Yates's correction (Parker, 1973).

RESULTS

The results of the GC experiment show that the developing syconia of *Ficus ingens* do emit a number of gaseous compounds, and that this emission is related to the size of the syconia. The number of peaks appearing in the different size classes also varies, but a general pattern seems to be followed. The increase in the number of peaks within these classes may be due to isomerization of the compounds, decay of the compounds, or genuine syconial products. These results are shown in Fig. 1a–e.

Fig. 2 is a histogram of the number of peaks in each size class as well as the control (air from an empty bottle plus solvent).

The results of the census of the wasps inside the syconium are presented as histograms (Fig. 3a–c). As may be expected, the 9 mm size class produced the most wasps per syconium. The results of the 6 mm class have not been presented as no wasps were found in any 6 mm syconia. On each of the bar charts, the mean (X) and sample size (n) has been shown.

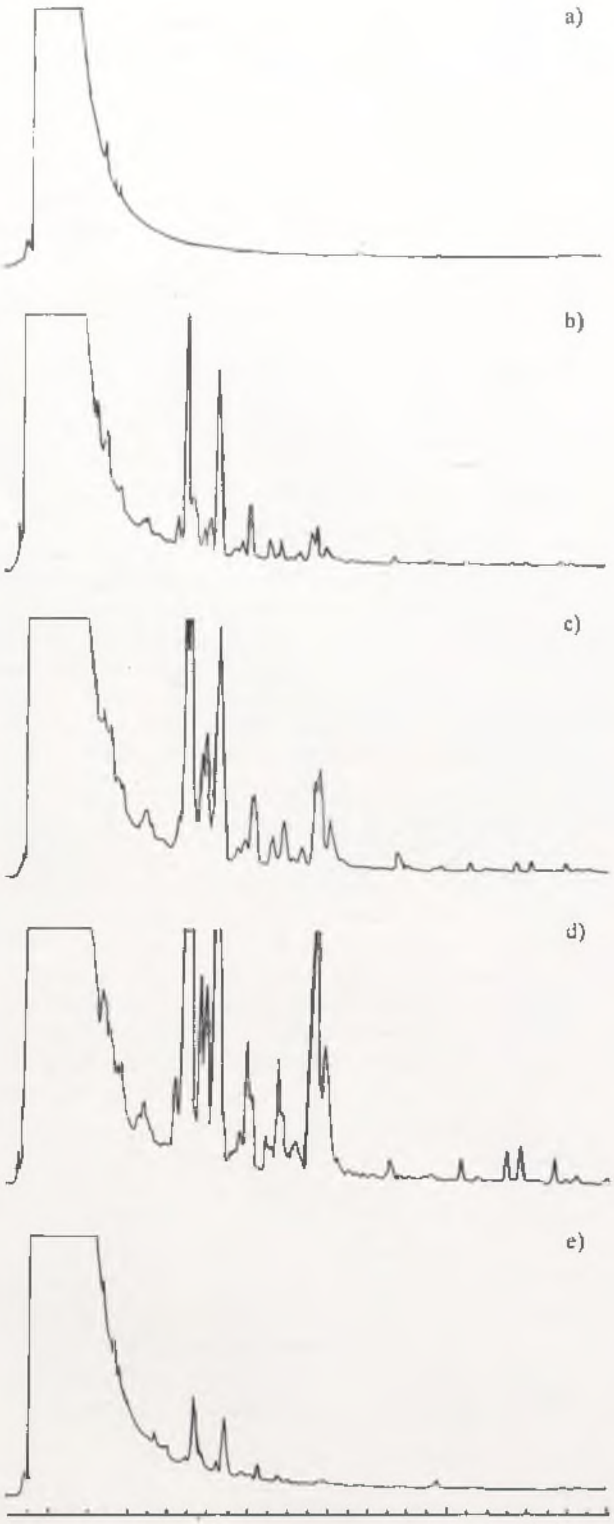

FIG. 1. — The integrated printouts from the GC runs of the control and the syconial size classes 6 mm to 9 mm. a, control; b, 6 mm syconia; c, 7 mm syconia; d, 8 mm syconia; e, 9 mm syconia. Starting temperature: 40 degrees held for 1 minute, increasing at a rate of 1,3 degrees per minute for 30 minutes. Integrator parameters: Attenuation = 0, chart speed = 0,5 cm/s, peak width = 0,10, threshold = 4 and area of rejection = 10 000. Scale: 1 unit = 1 minute elution time. Total run time: 30 minutes.

The results of the SEM studies showed that the female *Platyscapa soraria* had thoracic and coxal corbiculae and these were found to be full of pollen in those wasps that were captured outside the syconia, whereas those wasps that were removed from the syconia just after they had hatched had empty pockets.

The results of the presence-absence of wasps in syconia with exudate are presented in Table 1. The data proved to be significant at the 95% confidence interval.

In all the collections of wasps made, no parasitic wasps were found: only the true pollinator, *Platyscapa soraria* Wiebes was present.

DISCUSSION

The gas chromatography method requires scrupulous cleanliness of equipment. This aspect was therefore rigidly controlled. Vitiation of results can arise from the fact that a GC machine is not easily transportable and therefore cannot be taken to the study site. This restriction to a laboratory means that samples have to be cut from the plant and taken to the laboratory. This immediately limits the life of the samples, as they are now entirely dependent on nutritional supplies in the twig. This in turn means that, sooner or later, the plant material will start to decay, and therefore there is a source of unwanted decay products. For the purposes of this study it is assumed that, as the samples were all analysed on the same day as they were removed from the tree, this decay had not yet started.

This removal may also cause the figs to respond by emitting a different set of compounds, or to stop emitting some and emit others, etc. As far as the analysis by GC is concerned, the unnatural starting temperature of the GC oven (40°C) may cause the breakdown or modification of thermolabile compounds. For the adequate interpretation of these results, these problems will have to be kept in mind, but assumed not to happen.

The GC results indicate that a number of volatile compounds are emitted by the syconia, and that this emission is related to the size (and therefore age) of the syconia. The syconia of the 8 mm size class emitted the most compounds, and the others, especially those in the 9 mm class, emitted less (Fig. 2).

The number of peaks does not necessarily indicate the maximum amount of activity, as the increased number may be due to isomerization of some of the products, or possibly, decay products. However, as these extra peaks appear in the general region of the main peaks, and not in totally new regions of the chromatograph, it is possible that these peaks have

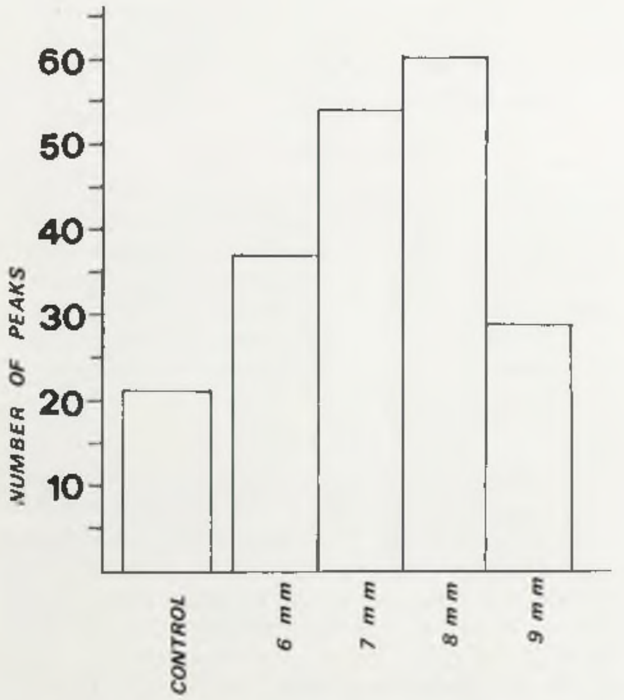

Fig. 2. — A histogram showing the number of peaks which were detected by the gas chromatograph for the control (air from an empty bottle) and each syconial size class.

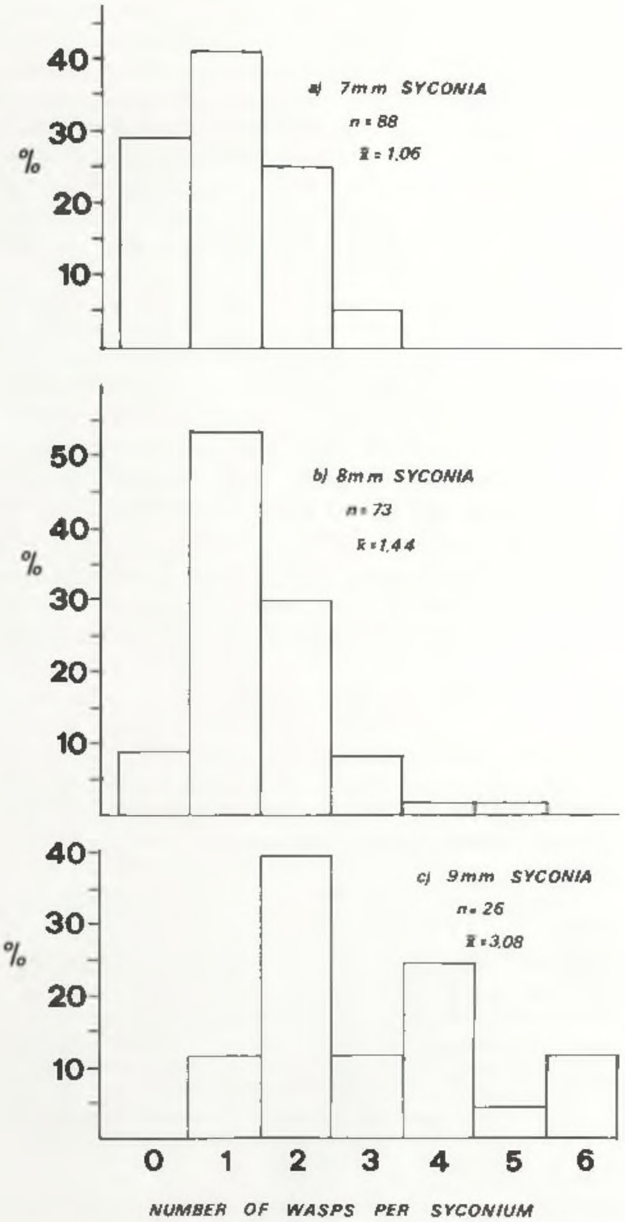

Fig. 3. — Histograms showing the number of wasps per syconium against frequency, expressed as a percentage of syconia sampled. a, 7 mm size class; b, 8 mm size class and c, 9 mm size class. The 6 mm size class had no wasps present in any syconia sampled. The mean (x) and sample size (n) are shown.

been separated out by the column due to increased production, so indicating peak activity.

The strong association between wasp activity and the presence of the volatile compounds is reflected in the visitation data. The 6 mm size class had not yet been visited by wasps, but was starting to produce some of the volatile compounds. This nonvisitation may be because the wasps could not detect the compounds in sufficient quantities, or else because the ostiolar scales had not yet loosened sufficiently to allow the entry of the wasps.

These scales are thought by Janzen (1979a) to have assumed the function of pollinator discrimination, and Ramirez (1974) has shown a strong relationship between the shape of the wasp's head and the ostiolar structure. The mean number of wasps per fig in the 7 mm and 8 mm size classes (as shown in Fig. 3a & 3b) indicates a tendency for the wasps to search for unentered syconia. Janzen (1979c) suggests that this may be a mechanism to reduce competition between the offspring of different mother wasps. How the wasp discerns whether a syconium has been entered or not is not known. It has, however, been reported (Janzen 1979a, 1979c) that after the wasp enters the syconium, a fluid is secreted by the fig which fills the syconium. This fluid is thought to have antibiotic and antifungal properties, as decomposition of the wasps's bodies is seldom found.

It was observed that syconia of *Ficus ingens* exude a fluid, but that the syconial cavity is not filled. This fluid is exuded from the osteole of the syconium, which dries to form an effective seal against further wasp entry. The stimulus for the production of this fluid is not clear, but may be as a result of injury to the syconium caused by the wasp. The time span between wasp entry and fluid production was not measured.

The results of the census of wasps in syconia with and without exudate showed that the presence of exudate was a good indication of wasp entry, at the 95% confidence limit as shown in Table 1. The time span in which the wasps are able to enter the syconium starts at the time the osteolar scales loosen, and ends either when the production of volatiles ceases, or when the syconia exude a fluid which blocks up the osteole. In this time-span any number of wasps may enter the syconium.

TABLE 1.—Presence or absence of wasps for syconia with and without exudate at the ostiole. This table is presented in the form of a 2 × 2 contingency table. A Chi-square test performed on this table showed the Chi-square value to be 4,847 (with Yates's correction). As this is greater than the expected value of 3,84 (at the 95% confidence level), the null hypothesis (that there is no relationship between visitation and the presence of the exudate) must therefore be rejected

	Wasps	No wasps	
Exudate	36	0	36
No exudate	11	3	14
	47	3	50

The results of the wasp census (from the syconia used in the GC experiment) indicate that wasps prefer to enter previously unentered syconia. This is indicated by the value of the mean number of wasps per syconium in the 7 and 8 mm size classes (Fig. 3a & 3b). This observation duplicates that of Janzen (1979c), who observed similar behaviour in other fig species. The mean number of wasps per syconium in the 9 mm size class would probably be a reasonable indication of the final number of wasps that enter a *Ficus ingens* syconium (3–4 per syconium, as shown in Fig. 3c). It was also noted that syconia in this size class had much larger ovaries, as the wasps had already laid their eggs, and the ovaries had then begun to develop into galls, as all wasp-infested female fig flowers do (Janzen 1979b). This resulted in the syconium becoming much more 'woody' or tough, as the galls developed. Ramirez (1970) and Janzen (1979c) observed that if a syconium was not entered by a wasp, it aborted and fell from the tree. No data on this aspect were collected for *Ficus ingens*.

CONCLUSION

The co-incidence of the production of numerous volatile substances and wasp visitation to the syconia of the size and age at which the volatiles were produced indicate that *Ficus ingens* attracts its specific wasp pollinator by using odours. As most of the fig species have only one legitimate wasp pollinator species, it is to be expected that different fig species produce different compounds, especially if they exist in sympatry with one or more other fig species. It should therefore be possible to 'fingerprint' each *Ficus* species by its characteristic signature of volatiles, when analysed by using gas chromatography.

Further research in this field may include the isolation and identification of these compounds, and the behavioural and physiological analyses of the wasps's reactions to the components of the volatile fraction, to determine which compounds release a behavioural reaction. It may be found that the wasps need more than one of these compounds before the searching behaviour is elicited.

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UITTREKSEL

Die verwantskap tussen die vy Ficus ingens *(Miq.) Miq. en sy bestuiwer, die perdeby* Platyscapa soraria *Wiebes, is bestudeer. 'n Meganisme van aantrekking van die bestuiwer word bespreek en bevestiging verkry deur die gebruik van gas chromatografie om die aanwesigheid van vlugtige verbindinge aan te toon. Dit word bewys dat hierdie verbindinge gedurende die vroulike stadium van die vy se blomsiklus vrygestel word. Hierdie data word met die gedrag van die bestuiwer en data betreffende besoeke, verkry van waarnemings in die veld, gekorreleer.*

REFERENCES

GALIL, J., 1977. Fig biology. *Endeavour* 1: 52–56.

GALIL, J. & EISIKOWITCH, D., 1968a. Flowering cycles and fruit types of *Ficus sycomorus* in Israel. *New Phytol.* 67: 745–758.

GALIL, J. & EISIKOWITCH, D., 1968b. On the pollination ecology of *Ficus sycomorus* in East Africa. *Ecology* 49: 259–269.

GALIL, J. & EISIKOWITCH, D., 1974. Further studies on pollination ecology in *Ficus sycomorus*. II. Pocket filling and emptying by *Ceratosolen arabicus* Mayr. *New Phytol.* 73: 515–528.

GROB, K., 1975. The glass capillary column in gas chromatography. A tool and a technique. *Chromatographia* 8: 423–433.

HILL, D. S., 1967, Figs (*Ficus* spp.) and fig-wasps (Chalcidoidea). *Jnl. Nat. Hist.* 1: 413–434.

JANZEN, D. H., 1979a. How to be a fig. *A. Rev. Ecol. Syst.* 10: 13–51.

JANZEN, D. H., 1979b. How many babies do figs pay for babies? *Biotropica* 11: 127–129.

JANZEN, D. H., 1979c. How many parents do the wasps from a fig have? *Biotropica* 11: 127–129.

PARKER, R. E., 1973. *Introductory statistics for biology*. London: Arnold.

RAMIREZ, W., 1970. Host specificity of fig wasps (Agaonidae). *Evolution* 24: 680–691.

RAMIREZ, W., 1974. Coevolution of *Ficus* and Agaonidae. *Ann. Mo. bot. Gdn* 61: 770–780.

WIEBES, J. T., 1979. Coevolution of figs and their insect pollinators. *A. Rev. Ecol. Syst.* 10: 1–12.

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Analysis of the size and composition of the southern African flora

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Keywords: endemic species, flora, phytogeography, plant families, species diversity

ABSTRACT

The southern African flora has been surveyed for the first time at species level in the *List of Species of Southern African Plants* (Gibbs Russell *et al.*, 1984). The numbers of taxa recorded for southern Africa are compared to the numbers reported from other parts of Africa, and the largest families in each area are listed and compared. The species richness of southern Africa is compared to that of other parts of the world. The numbers of genera, species and infraspecific taxa are given for each family in the southern African flora, and compared to previous counts by Dyer (1975, 1976) and Goldblatt (1978).

INTRODUCTION

The remarkable diversity and high level of endemism in the southern African flora has been reported by a number of authors, notably Adamson (1938), Weimarck (1941), Levyns (1964) and Good (1974), and has been discussed in great detail by Goldblatt (1978). Until recently there was no modern inventory of the taxa on the subcontinent, but the publication of the *List of Species of Southern African Plants* (Gibbs Russell *et al.*, 1984) now provides for the first time a complete coverage of the entire flora. The components of the southern African flora can now be precisely analysed and comparisons can be made with floras of other parts of Africa and with previous estimates of the southern African flora. The relative importance of present studies can be assessed, and future work on the *Flora of Southern Africa* can be planned with a more accurate idea of the magnitude of the task.

METHODS

The numbers of taxa reported here for southern Africa are taken from the PRECIS list of 30 June 1984, and some modifications have been made since the first edition of the *List of Species* went to press in October 1983. The taxa in the southern African flora were counted by computer, and have been verified by a manual count of the *List of Species*. Counts for other Floras in Africa were made by hand, as described in Gibbs Russell (1974).

Because the Floras considered for this study differ in the delimitation of families and in the level of recognition of species and infraspecific taxa, it was necessary to adopt a uniform treatment in order to compare them. Families are treated *sensu lato*, and the genus and species counts for the segregate families are added to give a single count in these cases. Notable examples of families treated in this way are Aizoaceae (includes Mesembryanthemaceae), Asclepiadaceae (includes Periplocaceae), Campanulaceae (includes Lobeliaceae), Fabaceae (includes Caesalpinioideae, Mimosoideae and Papilionoideae), Liliaceae (includes Alliaceae, Asparagaceae, Asphodelaceae, Colchicaceae, Dracaenaceae, Eriospermaceae and Hyacinthaceae) and Scrophulariaceae (includes Selaginaceae). The alternative to a broad acceptance of these families would have been to split them apart in Floras which treat them as units. This was not done because an object of the study is to convey an overall picture of the southern African flora in relation to the floras of other parts of Africa, and it was therefore more consistent to accept these families in the broadest sense.

It was also necessary to adopt as far as possible a uniform treatment of the lower taxa, especially those of infraspecific rank. Because one author's species may be another author's subspecies or variety, the total species number for different accounts of the same group can differ considerably, and are not readily comparable. For this reason, combined totals of species plus infraspecific taxa were used on the tables below for purposes of comparison. For example, *Crassula* was revised for southern Africa by Tölken (1977), and this revision is followed in the *List of Species*. There are now 237 taxa, including 142 species with 47 subspecies, 39 varieties and 9 recognized hybrids. Examination of his treatment shows that the great majority of taxa now accepted by him at the infraspecific level were originally recognized as species. Of the 29 species given for the Cape Peninsula by Adamson & Salter (1950), 9 are accepted at the infraspecific level by Tölken (1977). If these treatments were compared at the level of species, 30% of the *Crassula* species in Adamson & Salter (1950) would not be counted in the *List of Species* that follows Tölken, thus giving the Adamson & Salter (1950) count a falsely high comparative value.

Just as the different Floras considered here cannot be directly compared because of differences in treatment, so the different parts of the *List of Species* itself vary widely in their ranking of taxa because the list is the result of taxonomic judgement by numerous individuals made over at least 80 years. A recently revised group such as *Crassula* may contain fewer species and more infraspecific taxa, while a group greatly in need of revision, such as the entire family Mesembryanthemaceae, presently has a great many species that will probably be reduced when they are critically studied. The numbers of species

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