

## Genetical studies of nectar and pollen production in sunflower

F Vear<sup>1</sup>\*, M Pham-Delegue<sup>2</sup>, D Tourvieille de Labrouhe<sup>1</sup>, R Marilleau<sup>2</sup>,  
Y Loublier<sup>2</sup>, M le Métayer<sup>2</sup>, P Douault<sup>2</sup>, JP Philippon<sup>1</sup>

<sup>1</sup> INRA, Station d'Amélioration des Plantes, Domaine de Crouelle, 63039 Clermont-Ferrand Cedex;

<sup>2</sup> Laboratoire de Neurologie Comparée des Invertébrés, INRA-CNRS (UA 1190), 91440 Bures-sur-Yvette, France

(Received 24 January 1989 ; accepted 24 January 1990)

**Summary** — The composition and quantitative characteristics of nectar and pollen production were studied in 2 series of parental lines and the hybrids obtained from a factorial cross. Nectar analysis by gas chromatography and enzyme electrode technology were compared; the former was found to be the most satisfactory. Nectar sugar composition was very stable, with 52% fructose and 48% glucose. Sucrose was rarely present in trace amounts. Significant differences were found in the volume of nectar per floret, percentage dry matter and energy value per floret. Heritable effects appeared most clearly for nectar volume per floret. The number of pollen grains per floret varied between plants but there were significant genotypic and parental effects. Possible relations between nectar and pollen production and agronomic characteristics are discussed.

**sunflower / nectar / pollen / analysis / heredity**

**Résumé** — Études de la variabilité génétique de la production de nectar et de pollen chez le tournesol. La composition et les caractéristiques quantitatives des productions de nectar et de pollen ont été étudiées sur 2 séries de lignées de tournesol et les hybrides issus de leurs plans factoriels de croisement (6x6 et 3x3). Des analyses du nectar par chromatographie en phase gazeuse et par une méthode électrolytique ont été comparées. Si la corrélation entre les valeurs moyennes par génotype mesurées par les 2 méthodes est significative, celle entre les valeurs par plante ne l'est pas, surtout parce que la méthode électrolytique fait apparaître des concentrations de saccharose qui sont inexactes selon les analyses en chromatographie (tableau I). Ainsi seule la chromatographie peut être utilisée. La composition des sucres du nectar est très stable, avec aucune différence significative entre 12 lignées et leurs 36 hybrides (tableau II). Il y a environ 52% de fructose et 48% de glucose. Le saccharose est rarement présent et en faible quantité. Il existe des différences significatives entre génotypes en ce qui concerne le volume de nectar par fleuron (tableau III), le pourcentage de matière sèche (tableau IV) et la valeur énergétique par fleuron (tableau V). Le volume de nectar par fleuron est la variable pour laquelle les effets héréditaires paraissent les plus nets; une lignée, GH, produisant 0,26 µl par fleuron, donne des hybrides avec en moyenne jusqu'à 0,31 µl par fleuron. Alors que les autres génotypes produisent en moyenne 0,10 µl /fleuron. Le nombre de grains de pollen par fleuron varie entre plantes d'un même génotype. Néanmoins il y a des effets génotypiques (tableau VI) et parentaux (tableau VII) pour ce caractère. Le nombre de grains par fleuron moyen varie de 25 218 à 42 087 selon le génotype. Aucune relation avec la résistance ou sensibilité au Sclerotinia n'apparaît en ce qui concerne les productions de nectar et de pollen (tableau VIII). Il y a une tendance pour que les génotypes produisant moins de pollen soient un peu plus riches en huile, mais la corrélation n'est pas significative.

**tournesol / nectar / pollen / analyse / hérédité**

### INTRODUCTION

Pollen and nectar provide nourishment for organisms ranging from micro-organisms to man. However, the primary importance of pollen is as a carrier of the male gametophyte, so that all use of pollen as food is secondary and represents

pollen theft. In contrast, nectar clearly has no other function in angiosperm flowers than to provide food in plant-pollinator relationships.

It is likely that natural selection has tended to produce a food reward for cross-pollination. The relationship between sunflowers and their pollinating insects is a model of great interest.

\* Correspondence and reprints

Sunflowers are preferentially cross-pollinated by insects, mainly honey bees (Macgregor, 1976; Radford *et al*, 1979) and other apoids such as bumble bees (Freund, Furgala, 1982). Both plant and insect benefit from this relationship: sunflower varieties are  $F_1$  or 3-way hybrids produced using cytoplasmic male sterility; hybrid seed production depends on the transport of pollen by bees from the male parent to the male sterile female parent (Parker, 1981a). The hybrids themselves are bred for self-fertility but even for the most self fertile genotypes, it has been demonstrated that cross pollination by insects induces a notable increase in seed yield and oil content (Furgala *et al*, 1979) as well as more rapid seed set and thus improved earliness (Madeuf, unpublished data).

In parallel, sunflower floral structure is particularly adapted to attract honey bees. They have a strong aroma (Etievant *et al*, 1984), large ligulate florets (Harbourne, Smith, 1978), abundant nectar secretion with large quantities of sugar (Tepedino, Parker, 1982). There have been many studies of nectar secretion and sugar content. By far the largest dry weight of nectar is sugar, but various workers have demonstrated the presence of proteins, lipids, alkaloids and other compounds (Baker, Baker, 1976) which are probably of great importance in nutrition but represent less than 0.03% of dry weight.

For the majority of nectars, the sugar content is constituted by glucose, fructose and sucrose. It is known that their relative proportion in nectar is characteristic according to family but tends to remain constant for a given species (Wykes, 1952). In sunflower it had been reported that differential distribution of honey bees between the parental lines of 2 hybrids was related to differences in nectar composition, and more precisely to the relative amounts of sucrose (Pham Delegue *et al*, 1985; Fonta *et al*, 1985). However, it remained to determine whether these differences existed in a wide range of genotypes and whether they were heritable.

Pollen grains contain a wide range of amino acids, both in the free state and as protein, as well as lipids, carbohydrates and vitamins (Stanley, Linskens, 1974). The reward to a flower visitor is usually pollen produced in excess of the amount directly needed in pollination. However, it is not clear whether pollen production is characteristic of a given genotype.

In addition, pollen and nectar could be of importance in the determination of certain agronomic characteristics. Firstly, 2 of the most important diseases attacking sunflowers, *Sclerotinia sclerotiorum* and *Botrytis cinerea* can

result from infections occurring on the floral surface during flowering, and especially during the period of pollen and nectar production (Says-Lesage, Tourvieille de Labrouhe, 1988). The nectar and pollen, rich in nutritive substances could favorise spore germination and mycelial growth, and thus disease development. Secondly, Vear (1984) showed that male sterile plants have slightly higher oil contents and yields than comparable male fertile plants and suggested that this could be related to the saving in energy normally used for pollen production. Renard *et al* (1987) observed the same effect in rape-seed. Limitation in resources is an important aspect of pollen production and utilisation biology, although generally natural populations producing pollen are studied (Stanton, Preston, 1988), which is rather different from comparison of male sterile and male fertile plants.

This paper describes studies of the heredity of pollen and nectar production using different analytical methods, and the relations between these characters and *Sclerotinia* susceptibility and oil content.

## MATERIALS AND METHODS

### *Sunflower genotypes*

Two series of parents and  $F_1$  hybrids from factorial crosses were studied. The parental lines were chosen to represent a wide range of origins and to cover the known range of *Sclerotinia* resistance and susceptibility (Vear and Tourvieille de Labrouhe, 1988). In most cases, pollen and nectar production had not been studied before. The lines below are given in order of increasing susceptibility to *Sclerotinia*.

(a) 1985: 6 x 6 Female parents : SD, B11A3, F10, 62, GU, GH  
Males parents : PAC1, HA61, PRS5, RHA274, V135, RHA266

(b) 1986: 3 x 3 Female parents : B11A3, F10, GH  
Male parents : HA61, RHA266, V135

The female parents B11A3 and F10 have genic male sterility, the others cytoplasmic male sterility, but of course, for the studies of inbred lines, the male fertile forms were used. Of the male parents, only HA61 and V135 were not restorers.

### *Nectar analysis*

#### Sampling

The samples collected in 1985 were obtained on a single pooled sample from several plants, using the number of florets of the internal ring required to provide 10 ml of nectar. In 1986, 3 repetitions (plants) of each genotype (except the late flowering F10, F10 x Rha 266, F10 x V135) were collected individually to provide both quantitative and qualitative data.

The samples were obtained using graduated glass micropipettes (internal diameter 1 mm, volume 5 ml) in florets at anthesis on capitula during the first half of flowering. The capitula were covered with paper bags 24h before sampling, in order to obtain homogeneous evaporation conditions and to avoid visits by insects. The samples were conserved at  $-25^{\circ}\text{C}$  until chemical analysis.

### Gas chromatographic analysis

This technique is sufficiently precise and sensitive to analyse small quantities of nectar, in the region of 2 ml. The method was developed by Marilleau *et al* (in preparation) from a technique of Brobst and Lott (1966), used by Bosi (1973) for analysis of partially dehydrated nectars which permits determination only of the relative percentage of sugars in the dry matter. It was completed, following Black and Bagley (1978) by the introduction of an internal standard permitting calculation of the real quantities of sugars present.

#### Sample preparation

The samples were dehydrated during 1 night, under partial vacuum, in the presence of  $\text{P}_2\text{O}_5$ , then dissolved in a control solution of 2  $\mu\text{g}$  of triphenyl ethylene / $\mu\text{l}$  pyridine. The dilution volume varied according to the quantity of nectar obtained. When only 1 to 1.5 ml was available, the dilution volume was 0.5 ml, otherwise it was 1 ml.

0.45 or 0.50 ml of hexamethyl disilazine were added, giving a complete replacement of the free OH groups of sugars by trimethylsilyl groups (Buzon *et al*, 1968) to permit satisfactory separation of the sugars along the column. This was followed by addition of 0.05 to 1 ml of trifluoroacetic acid which fixes water molecules still present. The final dilution volume was 2 ml for large quantities of nectar and 1 ml for smaller quantities.

Triphenyl ethylene was chosen as an internal standard due to its migration duration, which does not interfere with the sugars studied; in addition, not being a sugar, it undergoes no structural changes.

#### Preparation of the reference mixture

A solution was prepared with 1 ml of the triphenyl solution described above, in which 2 mg of each reference sugar (sucrose, fructose and glucose) were dissolved. Then, as for the nectar samples, the sugars were transformed. All components then had a concentration of 1  $\mu\text{g}/\text{ml}$ . The reference mixture was injected after every 4 or 5 samples and at least once a day.

#### Chromatograph characteristics

- Girdel 3 000 chromatograph with automatic temperature control.
- Column of pyrex glass (length 3 m, internal diameter 2 mm).
- Stationary phase OV17 at 3% on chromosorb WHP.
- Column temperature from  $185^{\circ}\text{C}$  to  $280^{\circ}\text{C}$  with an increase of  $2^{\circ}/\text{min}$ .
- Detector temperature  $300^{\circ}\text{C}$ .
- Nitrogen (gas vector) pressure 2  $\text{kg}/\text{cm}^2$  flow : 20 ml/min.

- Integrator/recorder Delsi enica 10.
- Volume injected 2  $\mu\text{l}$ .

#### Parameters

The internal standard method of Black and Bagley (1978) was used: the migration coefficients ( $K_i$ ) of each sugar were calculated from the reference mixture.

$K = A's$  where:  $A's$  : area of the standard peak  
 $A'i$  : area of a given sugar peak

The concentration of each sugar is obtained by dividing the area under the peaks of the constituents of an unknown sample, multiplied by its migration coefficient, by the area of the peak obtained with a known quantity of the internal standard. The calculation is the following :

$$C_i = \frac{a_c}{a_s} \times k_i \times \frac{w_s}{w_c} \times 100$$

Where  $a_c$  = area of compound  $i$  in sample

$a_s$  = area of internal standard in sample

$w_s$  = weight of internal standard in sample

$w_c$  = weight of sample

This method takes into account changes in the efficiency of the column and avoids losses of time required for standard graph calculations.

Bolten *et al* (1979) suggest the adoption of a standard expression for sugar contents in nectar. Thus, sugar concentrations obtained from gas chromatography, expressed as mg sugar/100 mg nectar were converted using tables in the Handbook of Chemistry and Physics (1988) into mg sugar/100 ml nectar.

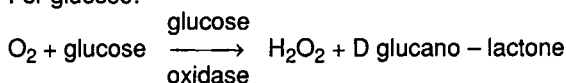
### Enzyme electrode technology

This technique was carried out on the samples collected in 1986. Successive measurements of each sugar of the nectar were provided by the YSI Model 27 Industrial Analyser (Bioblock). Samples were diluted in 100 ml of distilled water; 25  $\mu\text{l}$  of the solution were injected into a sample chamber containing a 5  $\mu\text{m}$  thickness of Nucleopore polycarbonate membrane material which has pores of nominally 0.03  $\mu\text{m}$  diameter. The rate of the following chemical reactions is limited primarily by the rate of this first diffusion step. Since diffusion is a physical process, much simpler and more predictable than enzyme kinetics, this results in improved linearity, calibration stability and freedom from enzyme inhibition errors. Once past the Nucleopore barrier, the sugar encounters a thin (1  $\mu\text{m}$ ) layer of immobilised enzymes of very high activity per unit volume.

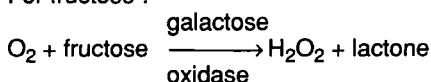
#### Glucose and fructose measurements

On the membrane, the following reactions occur almost instantaneously :

For glucose:



For fructose :

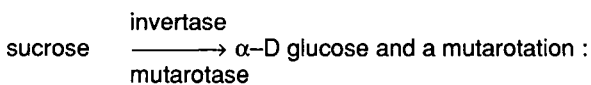


The hydrogen peroxide produced diffusing towards a probe anode is oxidised electrochemically. This gives rise to the probe signal current. The current flow at the anode is directly proportional to the hydrogen peroxide concentration and hence to the sugar concentration.

The readings, which represented the sugar free assay of the diluted sample in units of mg/100 ml, were recorded. The weight of sugar (expressed as mg/100 ml of nectar) was then obtained by multiplication of the reading with the dilution rate.

### Sucrose measurement

For the determination of sucrose in the presence of glucose, the glucose concentration of the sample is determined both before and after the complete hydrolysis (inversion) of the sucrose present to glucose. The sucrose is converted externally according to an inversion:



$\alpha\text{-D glucose} \longrightarrow \beta\text{-D glucose}$ . The sucrose concentration is calculated from the difference between the 2 glucose concentrations.

### Pollen counts

Pollen was collected from male fertile hybrids and the male fertile forms of the parental lines.

In 1985, the pollen counts were made on the 6 x 6 factorial, except for 4 hybrids and 2 inbred lines, with 4 counts per plant and 5 plants per genotype. Observations were completed in 1986 using 3 controls, observed both years (SD x PAC1, GH x RHA266, F10 x V135), to permit correction to the 1985 levels (multiplication of 1986 data by 1.067).

Following acetolysis and ultrasonic treatment, pollen grains were counted using a particles counter (Coulter Counter ZB Coultronics) according to the method described by Loublie *et al* (1986).

Ten mature, non-dehiscent florets were randomly collected from sunflower heads at mid-flowering. Samples of 10 florets were put in a laboratory vessel and ground in a few drops of distilled water in a glass homogeniser. Ten ml pure sulphuric acid were then added to act on the substrate for 24h; 90 ml of a 2% aqueous solution of triton were added and centrifuged until a single sediment was obtained. Each centrifugation was for 7 min at 3 000 rpm (about 700 g). Acetolysis was conducted following the method of Erdtman (1960). The sediment was dissolved in 5 ml ethanol at 95% and subjected for 15 min to an ultrasonic treatment with a frequency of 50 k/cycles/s. The particles were reconcentrated by centrifugation and rediluted in 50 ml Isoton. This solution was used to make 4 replicate measurements with the "Coulter Counter".

### Sclerotinia reaction

Reaction to *Sclerotinia* was assessed by 2 methods in 1986. Firstly by counts of the percentage of plants

attacked under natural conditions (with irrigation at flowering). This method was described in detail by Vear and Tourvieille de Labrouhe (1987). In a randomised block design with 46 plants per plot and 4 replicates, the plants were maintained with liquid water on the capitulum surface for at least 48h during flowering to favorise *Sclerotinia* infection. Environmental effects on symptom development were eliminated by comparison of results with those of plots of a control hybrid flowering at different dates. The results are thus expressed as an index (*Sclerotinia* Index 1) calculated by :

$$\frac{\text{Number of plants attacked of a given genotype}}{\text{Number of control plants attacked with same flowering date}}$$

Secondly, artificial infections were made with suspensions of ascospores during flowering (Tourvieille de Labrouhe and Vear, 1984). In this case, percentage infection is very high and resistance is measured by the delay in symptom appearance. As with semi-natural attack, environmental influences are eliminated by comparison with a control. The results are latency indexes (*Sclerotinia* index 2 in table II) calculated as :

$$\frac{\text{Number of days from infection to symptom appearance of a given genotype}}{\text{Number of days from infection to symptom appearance in controls with same flowering date.}}$$

Thus, for natural attack, higher figures indicate susceptible genotypes, whereas on the contrary, for the ascospore infection, the most resistant genotypes have the highest indexes (Vear and Tourvieille de Labrouhe, 1988).

### Oil contents

These are measured by NMR (Brucker Minispec 10) on 3 g. samples of seed at 4-6% moisture content.

### Studies of heredity

Genotypic differences were studied by analysis of variance : parental effects and interactions by factorial analysis and the relation between the phenotypes of inbred lines and those of their hybrids by correlations between results for inbred lines and the means of those obtained for their hybrids.

## RESULTS

### Nectar

#### Effect of analytical methods

The data (table I) obtained from gas chromatography and enzyme electrode technology for the nectar samples collected on the parents and F<sub>1</sub> hybrids of the 3 x 3 factorial cross of 1986 were compared. Samples from the female parent

**Table I.** Nectar production in 1986, measured by gas chromatography (C) and by the enzyme electrode technique (E), and oil contents for the inbred lines and hybrids of a 3 x 3 factorial cross.

Genotypes	% Fructose in DM		% Glucose in DM		% Sucrose in DM		% Dry matter		Vol nectar / floret	Energy val / floret	% Oil content
	C	E	C	E	C	E	C	E	( $\mu$ l)	(J)	
B11A3	51.5	51.6	48.5	48.4			65.3	74.0	0.04	0.44	38.2
GH	50.7	48.2	49.3	46.4		5.4	32.3	36.4	0.26	1.39	46.7
RHA266	50.9	45.8	47.3	40.0	1.8	14.2	66.3	60.8	0.11	1.17	48.8
HA61	50.3	51.5	49.7	47.1		1.4	73.3	70.3	0.08	1.06	43.4
V135	50.7	50.8	49.3	46.8		2.4	56.0	69.0	0.05	0.50	50.9
B11A3 x RHA266	51.9	53.3	48.1	41.6		5.1	50.0	77.3	0.09	0.73	40.5
GH x RHA266	54.0	48.5	46.0	47.5		4.0	58.0	59.6	0.10	0.95	53.0
B11A3 x HA61	51.4	50.8	48.6	43.1		6.1	68.0	79.6	0.04	0.50	42.9
GH x HA61	50.8	51.8	49.2	48.2			45.7	60.2	0.32	2.73	52.1
F10 x HA61	49.9	51.0	50.1	48.4		0.6	63.7	68.9	0.09	0.95	44.2
B11A3 x V135	52.5	49.2	47.5	45.9		4.9	59.5	73.7	0.06	0.67	47.3
GH x V135	51.2	50.3	48.8	44.5		5.2	53.0	65.6	0.29	2.68	49.9
F10 x V135	51.3	50.0	48.7	50.0			76.7	81.5	0.04	0.50	46.6
F genotypes	0.70 ns		1.01 ns				3.30 **		5.66 **	3.85 **	
lsd							19.23		0.12	1.14	

F10 and from the hybrid F10 x RHA266 were not available. Correlations between the values obtained from both techniques were calculated for dry matter concentrations, and for the relative proportions of each constitutive sugar.

For dry matter concentration, calculations were made on data recorded from 3 samples per genotype and on the mean values of these 3 samples. The relative proportions of sugars were compared considering only the mean values. The correlation coefficients for the dry matter concentrations were significant both when considering the mean values ( $r=0.78$ ,  $n=13$ ) and all samples ( $r=0.36$ ,  $n=39$ ). However, the correlation coefficients calculated for fructose ( $r=-0.17$ ,  $n=13$ ) and for glucose ( $r=0.33$ ,  $n=13$ ) were not significant, probably due to the high levels of sucrose detected by the enzyme electrode technique.

Since the enzyme electrode technology was not found very satisfactory and was carried out for one year only, further comparisons will only take into account data obtained from gas chromatography analysis.

## Composition

This was the first factor studied in 1985, since it was thought that qualitative differences could be more stable than quantitative observations. Table II gives the 1985 results of the 6 x 6 factorial. The 2 most important sugars are fructose and

glucose. Sucrose is only found in small quantities (up to 4.2% of the dry matter) in 5 hybrids and 1 inbred, with no relation between them: the inbred was RHA 266, the hybrids had HA61 or PAC1 as male parent. The relative percentages of fructose and glucose appear quite standard (48-54% and 46-52% respectively). Using the female parents as repetitions for the male parents, and reciprocally, no significant differences were shown. In table I, considering gas chromatography data, the overall 1986 results for the 3 x 3 factorial confirm that sucrose is present in traces only, the main sugars remaining fructose and glucose. Analysis of variance confirmed that there are no significant differences in the relative carbohydrate composition of the nectar produced by the genotypes studied. It may be considered as "sugar" as a whole. In contrast, significant variations in volume of nectar/floret, nectar dry matter content and energy value/floret appear. These quantitative characteristics were therefore studied in more detail.

## Quantitative characteristics

### Volume of nectar per floret

This character was studied in 1986 only (table I). Means for genotypes varied between 0.32  $\mu$ l/floret for GH x HA61 and 0.04  $\mu$ l/floret for the inbred B11A3 and the hybrids F10 x V135 and

**Table II.** Nectar production in 1985 and reaction to *Sclerotinia* by the inbred lines and hybrids of a 6 x 6 factorial cross.

<i>Genotype</i>	<i>% Fructose in DM</i>	<i>% Glucose in DM</i>	<i>% Sucrose in DM</i>	<i>% Dry Matter</i>	<i>Sclerotinia 1</i>	<i>Index 2</i>
<b>Inbred lines</b>						
B11A3	52.13	47.87		47	143	89
62	50.39	49.61		27	157	79
SD	52.17	47.83		57	161	127
GH	49.74	50.26		40	360	83
GU	50.79	49.21		57	219	78
F10	48.61	51.39		41	379	61
HA61	49.90	51.10		68	122	108
RHA274	48.04	51.96		53	196	103
RHA266	48.95	50.74	0.31	50	182	101
V135	50.28	49.72		53	146	81
PAC1	51.53	48.47		44	69	94
PRS5	50.04	49.96		46	123	93
<b>Hybrids</b>						
B11A3 x HA61	50.06	48.50	1.44	42	94	112
62 x HA61	48.21	47.57	4.22	62	88	99
SD x HA61	49.75	49.64	0.61	47	72	117
GH x HA61	51.04	48.65	0.31	31	132	116
GU x HA61	52.28	47.72		47	224	91
F10 x HA61	51.76	48.24		62	133	97
B11A3 x RHA274	51.99	48.01		52	49	101
62 x RHA274	50.32	49.68		70	105	97
SD x RHA274	50.90	49.10		48	108	105
GH x RHA274	49.91	50.09		35		98
GU x RHA274	50.53	49.47		39	246	83
F10 x RHA274	49.53	50.47		35	221	92
B11A3 x RHA266	51.77	48.23		47	254	92
62 x RHA266	50.73	49.27		39	201	83
SD x RHA266					155	100
GH x RHA266	50.90	49.10		48	233	83
GU x RHA266	53.15	46.85		41	258	102
F10 x RHA266	52.62	47.38		68		
B11A3 x V135	50.14	49.86		68		80
62 x V135	52.91	47.09		44	248	81
SD x V135	49.03	50.97		42		102
GH x V135	54.37	45.63		67	581	84
GU x V135	49.05	50.95		48	407	77
F10 x V135	50.17	49.83		26	270	73
B11A3 x PAC1	52.14	47.86		36	31	108
62 x PAC1	51.58	48.42		40	145	85
SD x PAC1	49.50	50.25	0.25	56	71	130
GH x PAC1	51.18	48.82		50	152	93
GU x PAC1	51.86	48.14		50	47	96
F10 x PAC1	50.01	49.99		39	35	86
B11A3 x PRS5	52.11	47.89		52	138	96
62 x PRS5	51.32	48.68		47	82	83
SD x PRS5	53.84	46.16		61	109	117
GH x PRS5	53.16	46.84		58	363	83
GU x PRS5	52.92	47.08		62	179	89
F10 x PRS5	50.83	49.17		57	158	88

B11A3 x HA61. The 2 factorial analysis gave similar results (table III): there is no significant difference between male parents, whereas among the females, GH differs significantly from B11A3 and F10. Certain interactions are significant. Comparison between the inbreds and their hybrids shows only a very slight tendency to heterosis, the mean volume of nectar produced

by the hybrids (0.13  $\mu$ l) being similar to that produced by the inbreds (0.11  $\mu$ l). The very large quantity of nectar produced by the inbred line GH appears heritable, for GH x V135 and GH x HA61 produce 2-3 times more nectar than the hybrids with B11A3 or F10 as female parent. However, the correlation between the 5 inbreds and the means of their hybrids, although quite

**Table III.** Analysis of parental effects concerning the volume of nectar/floret ( $\mu\text{l}$ ).  
Correlation inbred line – mean hybrid  $r = 0.70$  NS.

Genotype	Inbred line	Mean parental effects in hybrids	
		Factorial 2 F x 3 M	Factorial 3 F x 2 M
B11A3	0.04	0.06	0.05
GH	0.26	0.22	0.31
F10			0.06
RHA266	0.11	0.10	
HA61	0.08	0.18	0.15
V135	0.05	0.17	0.13
$F_F$		21.13 **	19.01 **
$F_M$		2.08 NS	0.38 NS
$F_r$		4.64 *	0.30 NS
$\text{lsd}_F$		0.07	0.08

high ( $r = 0.71$ ), is not significant, probably because 4 of the 5 lines are indistinguishable for this character.

#### Dry matter concentration

##### Effect of year

In order to determine whether the significant differences between genotypes observed in 1986 repeated 1985 results, an analysis of variance was made using the single 1985 value (table I) and the mean of the 1986 values (table II), for the 14 genotypes observed for both years. There was no significant year effect but no genotypic effects appeared either and the correlation between the 2 years was weak ( $r = 0.11$  NS). However the 1986 results with 3 repetitions gave an  $\text{lsd}$  of 19.2 (table I), about 30% of the mean concentration, and these confidence limits cover a large part of the variation between 1985 and 1986. 1985 may be considered as simply showing the variability which occurs on 1 repetition.

##### Heredity

The factorial analysis were made using only 1986 results. In the absence of the inbred line F10 and the hybrid F10 x RHA266, 2 analysis were carried out (table IV) and they gave the same results. As in 1985, no significant parental effects or interactions may be demonstrated. The 3 male parents all give hybrids with 50-60% dry matter and this is true also of B11A3 and F10. Only the hybrids with GH as female parent show a slightly lower dry matter concentration. When the values for inbreds and their hybrids are compared (table IV), no heterosis appears, concentrations are sometimes slightly lower in hybrids. There is no

significant correlation between inbreds and the means of their hybrids but GH has the lowest concentration of dry matter both as an inbred line and in its hybrids.

##### Energy value

This value was calculated by multiplying the weight of sugar/floret by the energetic yield provided by 1 mg of sugar (16.725 J) (Bolten *et al*, 1979). The results (table V) resemble those for nectar volume/floret with GH differing from the other genotypes, both as an inbred line and in its hybrids.

**Table IV.** Analysis of parental effects concerning the percentage dry matter content in nectar.  
Correlation inbred line – mean hybrid  $r = 0.02$  NS.

Genotype	Inbred line	Mean parental effects in hybrids	
		Factorial 2 F x 3 M	Factorial 3 F x 2 M
B11A3	65.3	59.2	64.3
GH	32.3	51.6	49.3
F10			68.5
RHA266	66.3	54.0	
HA61	73.3	55.8	59.5
V135	56.0	56.3	61.9
$F_F$		1.05 NS	3.19 NS
$F_M$		0.06 NS	0.14 NS
$F_r$		1.67 NS	0.86 NS

**Table V.** Analysis of parental effects concerning the energy value per floret (J).  
Correlation inbred line – mean hybrid  $r = 0.33$  NS.

Genotype	Inbred line	Mean parental effects in hybrids	
		Factorial 2 F x 3 M	Factorial 3 F x 2 M
B11A3	0.44	1.04	0.59
GH	1.39	2.11	2.70
F10			0.72
RHA266	1.17	0.84	
HA61	1.06	1.61	1.39
V135	0.50	1.67	1.28
$F_F$		12.37 **	10.09 **
$F_M$		1.63 NS	0.06 NS
$F_r$		2.26 *	0.17 NS
$\text{lsd}_F$		0.11	1.06

**Pollen****Differences between plants**

In all cases, for inbreds or hybrids, the differences between plants were highly significant (with F values of 15.2-40.7 for the inbreds and 12.4-1 268.7 for the hybrids with 4/12 df). In hybrids, for a general mean of 34 268 pollen grains/floret, the lsd varies from 500-1 000. Thus, the 4 counts on each plant show good repeatability, but non genetic factors cause variation between plants.

**Differences between genotypes**

Analysis of variance between genotypes, using the plant means, showed significant differences both between inbreds and between hybrids (table

VI) although with lower F values and higher lsd than between plants. Pollen grain number per floret varies from 25 218 (F10) to 42 087 (B11A3 x RHA274) with a mean of 35 570 grains/floret for hybrids and 32 843 grains/floret for inbred lines. Of the 17 hybrids for which comparison with the 2 parents is possible, 10 show heterosis.

As results were available for only 25 hybrids of the possible 36, 3 factorial analysis were carried out using different combinations to determine parental effects (table VII). In all cases, female, male and interaction effects were significant. Of the female parents, B11A3 always gave hybrids which produced a lot of pollen, F10 gave hybrids with significantly less. SD and 62 (table VI) were not significantly different. Considering the male parents, HA61 and PRS5 gave hybrids with a lot of pollen, PAC1 and RHA266 were intermediate

**Table VI.** Numbers of pollen grains per floret and oil contents of inbred lines and hybrids.

<i>Genotype</i>		<i>Pollen grains/floret</i>	<i>Oil content (%)</i>
<b>Inbred lines</b>			
B11A3		31 231	38.2
SD		29 821	41.5
GH		39 236	46.7
F10		25 218	43.9
HA61		29 652	43.4
RHA274		35 132	42.3
RHA266		26 387	48.8
V135		39 387	50.9
PAC1		31 682	34.0
PRS5		40 788	40.8
	F = 9.72 **	lsd = 5 144	
<b>Hybrids</b>			
B11A3	x HA61	40 863	42.9
F10	x HA61	36 958	44.2
B11A3	x RHA274	42 087	42.5
62	x RHA274	31 803	50.4
SD	x RHA274	29 275	42.8
GH	x RHA274	32 692	44.7
GU	x RHA274	28 194	46.9
F10	x RHA274	29 792	43.3
B11A3	x RHA266	37 585	40.5
62	x RHA266	34 298	52.8
SD	x RHA266	34 777	
GH	x RHA266	37 872	53.0
GU	x RHA266	35 144	46.0
F10	x RHA266	29 749	52.2
B11A3	x V135	30 379	47.3
F10	x V135	34 321	46.6
B11A3	x PAC1	38 466	41.3
62	x PAC1	39 084	45.1
SD	x PAC1	33 153	43.3
GH	x PAC1	40 396	46.5
GU	x PAC1	37 770	48.4
F10	x PAC1	32 282	42.7
62	x PRS5	38 464	47.8
SD	x PRS5	37 467	43.0
	F = 5.01 **	lsd = 5 595	



**Table VII.** Analysis of parental effects concerning the number of pollen grains per floret.

Genotype	Inbred line	Mean parental effects in hybrids		
		Factorial A	Factorial B	Factorial C
B11A3	31 231	39 379	37 876	39 632
SD	29 822	32 401		33 668
F10	25 218	30 607	32 620	
HA61	29 652		40 260	
RHA274	35 132	32 307	35 940	34 388
RHA266	26 397	34 904	33 667	35 553
V135	39 277		32 350	
PAC1	31 682	36 859	35 374	36 901
PRS5	40 788			38 774
F <sub>F</sub>		11.84 **	23.00 **	14.32 **
F <sub>M</sub>		12.28 **	4.13 **	4.20 **
F <sub>r</sub>		2.99 **	5.97 **	3.38 **
lsd <sub>F</sub>		1852	2203	2263
lsd <sub>M</sub>		2619	3483	2613
Correlation inbred line – mean hybrid <i>r</i>		0.40 NS	0.06 NS	0.38 NS

and not significantly different, hybrids with V135 had the least pollen, those with RHA274 varied according to the female parent.

As might be expected with significant interactions between parents, the phenotypic correlation between inbreds and the means of their hybrids was not significant (according to combination,  $r$  varied between 0.06 and 0.40  $N=7$ , NS). Nevertheless, it may be noted that F10 and SD always give relatively little pollen, B11A3 and PRS5 greater quantities.

### Relations with nectar production

Nectar dry matter production did not appear significantly correlated with pollen production in 1985 ( $r=0.08$ ,  $N=33$ ) or in 1986 ( $r=0.42$ ,  $N=10$ ).

### Relation of nectar and pollen with *Sclerotinia* reaction and oil content

#### Sclerotinia reaction

The results of *Sclerotinia* observations are given in table II. Table VIII gives the series of correlations established with 1985 and 1986 results for nectar and 1985 results for pollen. For nectar, when a wide range of genotypes was studied, no correlation appeared. However,

correlations between reaction of different genotypes to natural attack and their volume of nectar/floret and energy value are significant. This could be a biological relation but the significantly different nectar production by GH, a line very susceptible to *Sclerotinia*, may be the reason for these correlations. It would be necessary to study a wider range of genotypes to determine the true relation. In the case of pollen, no significant correlations with *Sclerotinia* reaction were shown, but the hybrids show a slight tendency to greater susceptibility when less pollen is produced.

#### Oil content

The oil contents of the inbred lines and hybrids studied for their pollen production are given in table VI. The correlation coefficients: pollen number – oil content,  $r=0.13$ ,  $N=11$  (inbreds) and  $r=-0.23$ ,  $N=24$  (hybrids) are not significant, although they show a slight tendency for genotypes producing less pollen to have higher oil contents.

The oil contents of the genotypes on which nectar production was studied are given in table I. The correlations with energy value/floret were  $r=0.51$  ( $N=13$ , all material) and  $r=0.60$  ( $N=8$ , hybrids only). Both results are just below the significance level, but again GH may be the preponderant cause. It may be noted that GH

**Table VIII.** Correlations between nectar or pollen production and *Sclerotinia* reaction.

		Nectar		Pollen			
		% DM 1985	% DM 1986	Vol/ floret	Energy val	Inbreds 1985	Hybrids 1986
<i>Sclerotinia</i> natural attack	<i>r</i>	0.14	-0.40	0.55*	0.51*	0.03	-0.27
	<i>N</i>	31	13	13	13	10	25
<i>Sclerotinia</i> ascospore test	<i>r</i>	0.04	0.08	0.19	0.30	0.08	0.19
	<i>N</i>	35	13	13	13	10	25

and, to a less extent RHA266, have high nectar and oil contents, whereas B11A3 has a low nectar and oil content.

## DISCUSSION

### *Nectar production*

#### Methodology

Various methods of nectar analysis are available.

\* The volume of nectar per floret may be determined from different collection methods. As an effect of collection method on estimation of nectar production was noted by Girnik (1976), who preferred micropipette collection to both centrifugation and washing, pipette collection was used in our work.

\* The concentration of total sugars in samples of several  $\mu$ l of nectar can be measured with a refractometer (Heinrich and Raven, 1972), or by summing the constitutive sugars as in our work.

\* The carbohydrate composition of nectar may be identified using paper (Scogin, 1979), thin-layer (Brewer *et al*, 1974) and gas-liquid (Bialeski and Redgwell, 1980) chromatographic techniques. In the present work, analysis were conducted firstly using gas chromatography described by Bosi (1973), adapted following Black and Bagley (1978). This method allowed a reliable determination of nectar sugars from small nectar samples but needed a complete derivatisation of the mono- and bi- saccharides before gas chromatography analysis, which involved a rather time-consuming procedure. In order to test another possibly more rapid method, the same nectar samples were analysed with enzyme electrode technology, usually applied for sugar analysis in foodstuffs.

The 2 methods gave equivalent results for dry matter but differed particularly in the

determination of the presence/absence of sucrose and the concentrations of this compound. Higher amounts of sucrose found from the enzyme electrode method appear to be related to the estimation procedure in which the quantity of sucrose is deducted from a double reading of glucose before and after inversion. In addition, it should be noted that galactase oxidase is a relatively non specific enzyme, and there may be substrates present in nectar samples which could interfere. Gas chromatographic analysis thus appear preferable for further analysis. The use of high-performance liquid chromatography may be suggested, since the development of this technique has allowed several authors to conduct rapid and reproducible determinations of nectar carbohydrate composition (Erickson *et al*, 1979; Freeman *et al*, 1983).

### Genetic variability

#### *Qualitative characteristics*

Analysis in 1985 and 1986 confirm that the 3 sugars that may be present in nectar are glucose, fructose and sucrose, as previously shown in sunflower, using the same technique (Bosi, 1973; Fonta *et al*, 1985) and using paper chromatography (Wykes, 1952). Bosi detected another sugar, raffinose, which may depend on the genotypes considered. However, although chosen for their genetic diversity, neither the group of 36 hybrids, nor the group of 9 hybrids showed any significant variation, with only trace quantities of sucrose in a few lines, and stable proportions of about 52% fructose and 48% glucose. Inbred lines comparable with the female parent of the hybrid Mariane, whose nectar contains up to 50% sucrose, reported by Fonta *et al* (1985), do not appear common. These authors suggested that it might be possible to characterise sunflower lines according to the

quality of their nectar. The present results suggest that by carbohydrate composition it would rarely be possible to do this. However, it must be kept in mind that many other nectar constituents may characterise sunflower genotypes. More particularly, the occurrence of amino acids, although the amounts are small in comparison with the sugars, may be significant nutritionally for bees.

#### *Quantitative characteristics*

The 1986 results indicate that some repeatable quantitative differences occur.

Sunflower nectar secretions are reported to vary from 0.02 to 0.16  $\mu\text{l}$  per floret (Tepedino and Parker, 1982). Some of the measurements reported here are higher than these values, perhaps due to the genotypes considered. There were significant differences between genotypes, and 1 inbred line, GH, had significantly more nectar than any of the others, both for *per se* values (0.26  $\mu\text{l}$ /floret) and for 2 of its 3 hybrids. Increased nectar production appears dominant in the case of GH x HA61 (0.32  $\mu\text{l}$ /floret) and GH x V135 (0.29  $\mu\text{l}$ ) but for GH x RHA266 (0.10  $\mu\text{l}$ ) the nectar level is close to that of the male parent RHA266 (0.11  $\mu\text{l}$ ) which with a moderate quantity of nectar both in the inbred line and in its hybrids (0.09 to 0.11  $\mu\text{l}$ ) also appears to contain some dominant genes.

It is difficult to advance further in the study of heredity for, with the exception of GH, the 5 other inbred lines are not significantly different. It would appear that genotypes producing a very large quantity of nectar are quite rare.

Total sugar concentration (dry matter) values from 30-50% are usually reported in sunflower (Burmistrov, 1965; Parker, 1981b). Although covering this range, in both 1985 and 1986, genotypes such as HA61 and F10 x V135 contained 70% or more. The results obtained in 1986 showed significant differences between genotypes but no significant parental effects. This confirmed the 1985 results where there were no parental effects either. However, taking the genotypes studied over the 2 years, the correlation was not significant. This provides an indication of the variability which may occur in quantitative measurements of nectar, if samples are not taken from plants under the same conditions.

Sunflowers thus appear to produce nectar in which a quite constant series of sugars are produced and in which the dry matter concentration does not vary considerably. Genotypes appear to differ most for the activity of their nectar secretion.

#### *Relations with agronomic characters*

There were no significant correlations between *Sclerotinia* resistance and nectar production in 1985. With the 3 x 3 factorial, correlations appear between *Sclerotinia* resistance and volume of nectar and energy value per floret and almost at a significant level between oil content and energy value per floret. However, it must be noted that these results depend largely on the inbred GH and its hybrids, which have large quantities of nectar, high oil contents and are susceptible to *Sclerotinia*. For all the other genotypes there does not appear to be any direct relation between nectar production and oil content or disease resistance. Nectar production does not appear to be a useful marker character for *Sclerotinia* resistance.

#### *Pollen production*

The 4 counts of pollen grain numbers per floret on any 1 plant showed that the method has good repeatability, as already reported by Loublie *et al* (1986). However, all the measurements on different plants and on different genotypes showed considerable variability. Hereditary effects can be distinguished (significant F genotype, F parental effects) but there are considerable interactions between parents of hybrids and environmental sources of variation (differences between plants of 1 genotype). It is difficult to explain these last variations, for differences in vigour between plants of 1 genotype do not appear to be involved, since the more vigorous hybrids frequently do not have more pollen than the inbred lines.

Between hybrid genotypes the number of grains varies from 28 to 40 thousand per floret. Significant differences mean that it would be possible to select genotypes with increased or decreased pollen production. However, although good pollen production is necessary in male parents and increased numbers of pollen grains appear to be slightly related to decreased *Sclerotinia* susceptibility (perhaps because they give more rapid fertilisation and so a reduced period of *Sclerotinia* susceptibility), the relation with oil is the reverse. A saving in energy use for pollen production could permit increased oil production. Veat and Tourvieille de Labrouhe (1988) report a significant negative correlation between *Sclerotinia* resistance and oil content in the material studied and variation in pollen number may be linked with this relation.

In conclusion, satisfactory methods have been developed for measuring nectar and pollen

production in sunflowers but the results of genetical studies are rather negative since there appears to be little variation in nectar production and, on the contrary, almost excessive variation in pollen production. There do appear to be some relations with agronomic characters, but these need further and wider studies and, in addition, may be favorable or unfavorable. No direct breeding programme concerning nectar or pollen production can be proposed until a further understanding of their physiology and roles in sunflower development, and possible interactions with environmental conditions, has been obtained.

## ACKNOWLEDGMENTS

The authors wish to thank Dr C Masson for helpful comments and criticisms.

## REFERENCES

- Baker I, Baker H (1976) Analyses of amino-acids in flower nectar of hybrids and their parents, with phylogenetic implications. *New Phytol* 76, 87-98
- Bielecki RL, Redgwell RJ (1980) Sorbitol metabolism in nectaries from flowers of *Rosaceae*. *Aust J Plant Physiol* 7, 15-25
- Black LT, Bagley EB (1978) Determination of oligo saccharides in soybeans by high pressure liquid chromatography using an internal standard. *J Ann Oil Chemist Soc* 55, 228-232
- Brewer JW, Collyard KJ, Lott CE (1974) Analysis of sugars in dwarf mistletoe nectar. *Can J Bot* 52, 2 533-2 538
- Bolten AB, Feinsinger P, Baker HG, Baker I (1979) On the calculation of sugar concentration in flower nectar. *Oecologia* 41, 301-304
- Bosi G (1973) Méthode rapide pour la détermination par chromatographie en phase gazeuse des glucides du nectar : technique de prélèvement du nectar et de préparation des éthers triméthylsilylis en présence d'eau. *Apidologie* 4, 57-64
- Brohst KM, Lott CE (1966) Determination of some components in corn syrup by gas-liquid chromatography of the trimethylsilyl derivatives. *Cereal Chem* 43, 35-43
- Burmistrov AN (1965) The melliferous value of some sunflower varieties. 20th Int Apic Congr Bucharest, 320-323
- Buzon J, Guichard N, Lebee J, Provot A, Serpinet J, Tranchant J (1968) *Manuel pratique de la chromatographie en phase gazeuse*. Masson, Paris, 357 p
- Erdtman G (1960) The acetolysis method : A revised description. *Sven Bot Tidskr* 54, 561-564
- Erickson EH, Thorp RW, Briggs DL, Estes JR, Daun RJ, Marks M, Schroeder CH (1979) Characterisation of floral nectars by high-performance liquid chromatography. *J Apic Res* 18, 148-152
- Etievant PX, Azar M, Pham-Delegue MH, Masson CJ (1984) Isolation and identification of volatile constituents of sunflower (*Helianthus annuus* L). *J Agric Food Chem* 32, 503-509
- Fonta C, Pham-Delegue MH, Marilleau R, Masson C (1985) Rôle des nectars de tournesol dans le comportement des insectes pollinisateurs et analyse qualitative et quantitative des éléments glucidiques de ces sécrétions. *Acta Oecol Oecol Appl* 6, 175-186
- Freeman CE, Reid WH, Becvar JE (1983) Nectarsugar composition in some species of agave (*Agavaceae*). *Madrone* 30, 153-158
- Freund DE, Furgala B (1982) Effect of pollination by insects and the seed set and yield of ten oilseed sunflower cultivars. *Am Bee J* 122, 648-652
- Furgala B, Noetzel DM, Robinson RG (1979) Observations on the pollination of hybrid sunflowers. Proc IV Int Symp Pollination, College Park 1978, 45-48
- Girnik D (1976) Productivité en nectar du tournesol. *Pchelovodstvo* 8, 10-11
- Harbourne JB, Smith DM (1978) Anthochlors and other flavonoids as honey guides in the *Compositae*. *Biochem System Ecol* 6, 287-291
- Heinrich B, Raven PH (1972) Energetics and pollination. *Science* 176, 597-602
- Loublier Y, Douault P, Pham-Delegue MH (1986) Méthode de comptage automatique des grains de pollen : étude de la production pollinique chez le tournesol (*Helianthus annuus* L), *Compositae*. *Apidologie* 17, 245-256
- MacGregor SE (1976) Insect pollination of cultivated crop plants. Agric Handbook US Dep Agric n° 496, Washington DC, 411
- Parker FD (1981a) Sunflower : abundance, diversity and seasonality of bees on male-sterile and male-fertile cultivars. *Environ Entomol* 10, 1 012-1 017
- Parker FD (1981b) Sunflower pollination: abundance, diversity and seasonality of bees and their effects on seed yields. *J Apic Res* 20, 49-61
- Pham-Delegue MH, Fonta C, Masson C, Douault P (1985) Étude comparée du comportement de butinage d'insectes pollinisateurs (abeilles domestiques (*Apis mellifera* L) et bourdons (*Bombus terrestris* L)) sur les lignées parentales d'hybrides de tournesol (*Helianthus annuus* L). *Acta Oecol, Oecol Appl* 6, 47-67
- Radford BJ, Rhodes JW, Nielson RGH (1979) Seed set in sunflower crops in the Central Darling Downs, Queensland. *J Anim Sci* 36, 133-139
- Renard M, Mesquida J, Pellan-Delorme R, Pelletier G, Morice J (1987) Pollinisation des hybrides mâles-steriles dans un système de culture mixte de colza. In : *Variabilité génétique cytoplasmique et stérilité mâle cytoplasmique*. (Bervillé A, ed). Les colloques de l'INRA 45, 281-292
- Says-Lesage V, Tourvieille de Labrouhe D (1988) Recherche des sites de pollution et d'infection de fleurons de tournesol, *in situ* par les spores de *Sclerotinia sclerotiorum*. *Inf Tech CETIOM* 102, 3-13
- Scogin R (1979) Nectar constituents in the genus *Fremontia* (*Sterculaceae*) : sugars, flavonoids and proteins. *Bot Gaz* 140, 29-31

- Stanley RG, Linskens HF (1974) *Pollen : Biology, biochemistry, management*. Berlin, Springer-Verlag, 307 p
- Stanton ML, Preston RE (1988) A quantitative model for evaluating the effects of flower attractiveness on male and female fitness in plants. *Am J Bot* 75, 157-159
- Tepedino VJ, Parker FD (1982) Interspecific differences in the relative importance of pollen and nectar to bee species foraging on sunflowers. *Environ Entomol* 11, 246-250
- Tourvieille de Labrouhe D, Vear F (1984) Comparaison de méthodes d'estimation de la résistance du tournesol à *Sclerotinia sclerotiorum* (Lib) de Bary. *Agronomie* 4, 517-525
- Vear F (1984) The effect of male sterility on oil content and seed yield in sunflower. *Agronomie* 4, 901-904
- Vear F, Tourvieille de Labrouhe D (1987) Le jugement des hybrides de tournesol vis-à-vis de l'infection naturelle des capitules par *Sclerotinia sclerotiorum*. *Inf Tech CETIOM* 99, 9-14
- Vear F, Tourvieille de Labrouhe D (1988) Relations between oil content and resistance to *Sclerotinia sclerotiorum* in sunflowers. Proc XIIth Int Sunflower Conf Novi-Sad, Yugoslavia, 110-114
- Weast RC (1988) *Handbook of Physics and Chemistry*. 68th ed (West RC ed) CRC Press Inc, Boca Roton, Florida, USA, 241 p
- Wykes GR (1952) An investigation of the sugars present in the nectar of flowers of various species. *New Phytol* 51, 210-215