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**A STUDY OF RAPESEED
(*BRASSICA NAPUS* L. VAR. *OLEIFERA* METZGER)
FLOWER NECTAR SECRETIONS**

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SUMMARY

Two nectar extraction techniques, micropipetting and centrifugation, were compared to establish a reliable basis for analysis and comparison of nectar production of rapeseed lines under selection. All tests used Kid cultivar. Apart from quantitative measurements for nectar production, nectar glucid composition was assessed by gas chromatography. Centrifugation provided larger quantities of liquid ($\times 4 - 6$), but total glucid content was not greater than for micropipetting. Centrifugation thus artificially diluted nectar and produced samples unrepresentative of those actually encountered by insects. Glucose and fructose fractions were very similar using either method. Micropipetting is therefore recommended for future work. Such sampling may provide the basis for selecting oilseed rape lines for nectar quantity and quality to optimize bee visits and cross pollination.

INTRODUCTION

Oilseed rape (*Brassica napus* L. var *oleifera* METZGER) is partially auto-gamous (70 %, SYLVEN, 1920 ; RIVES, 1957 ; MORICE, 1960 ; RUD-LOFF & SCHWEIGER, 1984), but its abundant nectar and pollen attract numerous insect pollinators. Various studies of rapeseed pollinating agents have indicated that the plant benefits from insect visitation (FREE & NUTTALL, 1968 ; WILLIAMS, 1978) despite the high efficiency of wind pollination (OLSSON, 1955 ;

MESQUIDA & RENARD, 1981, 1982). Insect pollination has more effect on plant phenology than on seed production (RENARD & MESQUIDA, unpublished data). Better understanding of the role of pollinating insects and factors affecting plant insect relations is of great importance to F1 hybrid seed production using male sterile lines.

Field experiments have shown that honeybees (*Apis mellifera* L.) are the main pollinators (TASEI, 1977 ; MESQUIDA & RENARD, 1978, 1979 (a), 1979 (b) ; RENARD & MESQUIDA, 1983). Moreover, foraging honeybees may show a preference for male fertile lines as opposed to male sterile lines (cytoplasmic « OGURA » type male sterility). The difference may reflect lower nectar secretion in these male sterile lines. Quality and quantity of nectar production have been shown to be significant for selective foraging behavior in honeybees (MASSON, 1983).

Therefore, reliable techniques for assessing nectar production of selected lines are needed if entomophilous pollination is to be fully assessed and exploited in selecting F1 hybrid seed parental lines. The objective is to equalize bee distribution between male sterile and male fertile lines in field crops.

This paper compares nectar collection from rape flowers using centrifugation versus glass micropipettes. In addition to quantitative analysis of nectar production, nectar glucidic component was also determined. The method used for glucidic composition analysis was derived from one previously applied to sunflower and oilseed rape (FONTA *et al.*, 1985 ; LE METAYER pers. comm.).

MATERIALS AND METHODS

Plant material

All experiments were done using a winter cultivar Kid, at the I.N.R.A. experimental station (Rennes, France). Samples were collected in the morning (8 h GMT) on freshly opened flowers, on two dates in April 1983. Plants in full flower were bagged 24 hrs prior sampling to prevent nectar collection by foraging insects.

Nectar extraction by centrifugation

Nectar was extracted from five flowers per plant on 15 April (first collection, 20 plants) and on 20 April (second collection, 10 plants). Sampling and centrifugation at 3 000 r.m. followed methods described by Bost (1973).

Nectar extract weight was calculated from the difference in weight before and after centrifugation.

Nectar extraction using micropipettes

Glass micropipettes (internal diameter 1 mm, volume 5 μ l.), were used to obtain samples from up to 5 flowers per plant from 20 plants in the first collection and from 10 plants in the second collection.

Volumes of nectar collected were read directly from micropipette scales, and samples were then weighed before freeze storage (-25°C) for later analyses.

After micropipette collection, up to 5 flowers from 6 plants were subjected to centrifugation in the second collection.

Analysis of nectar glucid composition

a) Sample preparation

Nectar was partially dehydrated under vacuum overnight in the presence of di-phosphorous pentoxide, after determining flower nectar weight. Trimethylsilylestere were prepared following BROHST & LOTT (1966) and BOSI (1973), the sample being dehydrated in pyridine (approximately 1 ml) with addition of 0.9 ml of hexamethyldisilazane and 0.1 ml of trifluoroacetic acid in that order. The solution was shaken for a few minutes, then left for 12 hours to ensure complete derivation of trisaccharides.

b) Preparation of standard reference solutions

Two mg of reference sugars (glucose, fructose or sucrose) were dissolved in 1 ml of pyridine, and dehydrated as for samples, so that sugars were then at $1\ \mu\text{g}/1$ concentrations.

c) Chemical analysis

A GIRDEL 75 gas chromatograph equipped with a pyrex glass column ($3\ \text{m} \times 2\ \text{mm}$) was used, filled with a stationary phase, type OV 17 at 3% on Chromosorb WHP. Column temperature was programmed from 185°C to 280°C , at $2^{\circ}\text{C}/\text{min}$. Detector temperature was 300°C . Carrier gas was nitrogen at $2\ \text{kg}/\text{cm}^2$ and a $24\ \text{ml}/\text{min}$ flow.

Detector signals were treated by a HOUSTON integrator recorder, recording speed $5\ \text{mm}/\text{min}$. Injected volumes were $2\ \mu\text{l}$. Standard solution was injected after each series of 4 or 5 samples.

d) Calculation of sugar proportions (following BOSI, 1973)

i. Proportion of dry weight attributable to each sugar

$$P_i \% = \frac{A_i f_i \times 100}{\sum A_i \times f_i}$$

A_i : peak area of a given sugar in standard solution ;

f_i : response coefficient calculated from a standard solution, relative to one of the reference sugars.

$$f_i = \frac{A_r}{A'_i}$$

A_r : area of reference sugar peak in standard solution ;

A'_i : area of sample sugar in standard solution.

ii. Total sugar concentration

Considered to be equal to nectar dry weight since amino acid and other nectar components represent less than 0.03% of nectar dry weight for most flower nectars (BAKER, cited by HEINRICH, 1975).

Nectar dry weight was obtained from

$$C\ \text{total}\ \% = \sum \frac{A_i}{A'_i} \times \frac{V}{P} \times 100$$

V = dilution volume ;

P = nectar weight prior to dehydration.

iii. Concentration of each sugar in nectar :

$$C_i \% = \frac{A_i}{A'_i} \times \frac{V}{P} \times 100$$

Data treatment

Effects of sampling method and sampling date, respectively, were analyzed using Student's t test after checking for applicability (DAGNELIE, 1970).

RESULTS*Nectar production*

Nectar weight per flower (mg) is shown in Table 1. Centrifugate sample volumes were always 4 to 6 times greater than for micropipettes. Sample volumes obtained using micropipettes were similar for the two collection dates, but differed significantly for centrifugated samples.

TABLE 1. — Mean weight (\pm standard error) (mg) of nectar/flower according to method and collection date, 1983.

Date		Micropipette collection	Centrifugation	Method effect
15 April	\bar{x}	0.80 (\pm 0.16)	4.77 (\pm 0.21)	***
	n	9	10	
20 April	\bar{x}	0.68 (\pm 0.16)	2.91 (\pm 0.11)	***
	n	6	9	
Date effect		n.s.	***	

Significance level as determined by Student's t-test

n.s. : not significant

* : $p < 0.05$

** : $p < 0.01$

*** : $p < 0.001$

Glucid content

The two principle sugars identified by chromatographic analyses were glucose and fructose.

● *Nectar sugar concentration*

Sugar concentration in nectar sampled by micropipette was between 40 % and 50 % (Table 2a). Concentration was about one fourth as high in centrifu-

gated samples, and this was also so for each sugar considered separately (tables 2b & 2c). There was no significant effect of sample date for either extraction method.

TABLE 2. — Mean concentrations (\pm standard error) (%) of nectar according to method and collection date, 1983.

	Date	Micropipette collection	Centrifugation	Method effect
a Total sugars or dry matter	15 April	41.08 (\pm 2.70)	11.21 (\pm 1.44)	***
	20 April	48.05 (\pm 5.27)	13.45 (\pm 0.88)	***
	Date effect	n.s.	n.s.	
b Fructose	15 April	19.61 (\pm 1.41)	5.57 (\pm 0.74)	***
	20 April	23.38 (\pm 2.58)	6.72 (\pm 0.42)	***
	Date effect	n.s.	n.s.	
c Glucose	15 April	27.47 (\pm 1.34)	5.65 (\pm 0.70)	***
	20 April	25.35 (\pm 2.96)	6.73 (\pm 0.46)	***
	Date effect	n.s.	n.s.	

Significance level as determined by Student's t-test

n.s. : not significant

* : $p < 0.05$

** : $p < 0.01$

*** : $p < 0.001$

TABLE 3. — Mean relative dry weight concentrations (\pm standard error) (%) of sugars, according to method and collection date, 1983.

	Date	Micropipette collection	Centrifugation	Method effect
a Fructose	15 April	47.29 (\pm 0.85)	48.48 (\pm 0.53)	n.s.
	20 April	48.65 (\pm 0.40)	49.90 (\pm 0.36)	*
	Date effect	n.s.	*	
b Glucose	15 April	52.71 (\pm 0.85)	51.53 (\pm 0.52)	n.s.
	20 April	51.35 (\pm 0.40)	50.10 (\pm 0.35)	*
	Date effect	n.s.	*	

Significance level as determined by Student's t-test

n.s. : not significant

* : $p < 0.05$

** : $p < 0.01$

*** : $p < 0.001$

- *Sugar proportions-dry weight*

Glucose (Table 3) content was higher than that of fructose by about 3 % – 4 %. There were slight differences in estimates for different sugars with date and method of collection, but no significant, except for centrifugated samples in the second collection.

- *Sugar content in nectar produced per flower*

Table 4 shows no significant difference between either dates or sampling methods for dry weights, or fructose or glucose contents. Total sugar weight in nectar produced per flower was approximately 0.4 mg (Table 4a).

TABLE 4. — *Mean weights (\pm standard error) (mg) of sugars in the nectar/flower, according to method and collection data, 1983.*

	Date	Micropipette collection	Centrifugation	Method effect
a Total sugars of dry matter	15 April	0.37 (\pm 0.06)	0.51 (\pm 0.07)	n.s.
	20 April	0.30 (\pm 0.04)	0.40 (\pm 0.03)	n.s.
	Date effect	n.s.	n.s.	
b Fructose	15 April	0.18 (\pm 0.03)	0.26 (\pm 0.03)	n.s.
	20 April	0.14 (\pm 0.02)	0.18 (\pm 0.02)	n.s.
	Date effect	n.s.	n.s.	
c Glucose	15 April	0.19 (\pm 0.03)	0.27 (\pm 0.03)	n.s.
	20 April	0.15 (\pm 0.02)	0.20 (\pm 0.02)	n.s.
	Date effect	n.s.	n.s.	

Significance level as determined by Student's t-test

n.s. : not significant

* : $p < 0.05$

** : $p < 0.01$

*** : $p < 0.001$

- *Centrifugation after micropipette collection*

Although an appreciable amount of liquid was extracted by centrifugation after micropipette sampling (Table 5a), the weight and concentration of sugars extracted were negligible (Tables 5b-g).

TABLE 5. — *Quantity and composition of nectars obtained from centrifugation following micropipette collection*

	Micropipette collection	Centrifugation following micropipette collection
a Nectar weight/flower (mg)	0.68 (± 0.16)	1.42 (± 0.36)
b Fructose weight in nectar/flower (mg)	0.14 (± 0.02)	0.01 (± 0.01)
c Glucose weight in nectar/flower (mg)	0.15 (± 0.02)	0.01 (± 0.01)
d Total nectar dry weight (mg)	0.29 (± 0.04)	0.02 (± 0.01)
e % fructose in nectar	23.38 (± 2.58)	0.67 (± 0.32)
f % glucose in nectar	25.35 (± 2.96)	0.53 (± 0.24)
g % dry matter in nectar/flower	48.05 (± 5.27)	1.20 (± 0.56)

Significance level as determined by Student's t-test

n.s. : not significant

* : $p < 0.05$

** : $p < 0.01$

*** : $p < 0.001$

DISCUSSION

The whole sugar fraction of nectar was obtained by micropipetting, although four to six times more liquid was extracted by centrifugation. Consequently, centrifugation diluted sugar fractions in samples relative to real content. An effect on collection method on estimation of nectar production was already noted for sunflower by MADEUF (pers. comm.) and GIRNIK (1976) who preferred micropipette collection to both centrifugation and washing. Other methods for measuring nectar secretion have been described. SWANSON and SHUEL (1949) considered a volumetric centrifugation method to be reliable for yield data with large scale sampling. LITZVEVA (1954) compared several nectar collection methods, and concluded that filter paper was least reliable (due to evaporation) as compared with pipette or washing methods. Water extraction methods seem to be seriously limited (von PLANTA, 1886 ; BONNIER,

1893 ; KENOYER, 1917), since extract sugar content may include sugars lixiviated from plant tissue cells. Most authors consider micropipette collection to be preferable, since it requires only the simplest equipment and is reliable (VANSELL *et al.*, 1942 ; VANSELL, 1943, 1944 ; SWANSON and SHUEL, 1949).

Proportions of fructose and glucose in Kid variety nectar were very similar, but no trace of sucrose was found, although the latter has been identified at low titres in other oilseed rape varieties (PERCIVAL, 1961).

Such differences in estimates of nectar glucid composition may be related to differences in sensitivity of gas chromatography techniques applied, but a varietal effect cannot be excluded. It might then be possible to detect and define nectar glucidic compositions specific to oilseed rape genotypes as suggested by FONTA *et al.* (1985) in reference to sunflower. Since nectar sugar content, and notably that of sucrose can be critical for bee foraging preferences (PHAM-DELEGUE *et al.*, 1985), screening of nectars of male fertile and male sterile lines for both sugar content and glucid composition may contribute to optimization of cross pollination.

In conclusion, even though no single method is satisfactory for all plants, nectar collection using micropipettes is preferred to centrifugation for study of rapeseed flower nectars. The latter provides larger sample quantities, but these are unrepresentative of nectar actually accessible to insects, and modification of chemical composition, due to lesion of plant tissues cannot be excluded. Micropipette nectar collection offers the best available sampling method, for assessing quantitative and qualitative aspects of nectar production as functions of genotype and phenological state. It may also be used to determine pedoclimatic influences on nectar secretion.

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RÉSUMÉ

ÉTUDE DES SÉCRÉTIONS NECTARIFÈRES DES FLEURS DE COLZA (*BRASSICA NAPUS* L. VAR. *OLEIFERA* METZGER)

La pollinisation du colza (*Brassica napus* L. var. *oleifera* METZGER), assurée en partie par le vent, dépend toutefois de l'action des insectes pollinisateurs, notamment dans le cadre de la production de semences hybrides F1. Chez cette plante, la faune pollinisatrice est principalement constituée d'abeilles

domestiques ; celles-ci manifestent des préférences génotypiques chez certaines lignées mâles-stériles qui pourraient être liées à des différences de sécrétions nectarifères. Afin de disposer d'une technique fiable d'estimation et de comparaison des productions nectarifères de différentes lignées en cours de sélection, deux techniques d'extraction des nectars, par centrifugation et par pipetage, ont été comparées pour les nectars de la variété Kid. Outre une mesure quantitative des productions nectarifères, une méthode d'analyse par chromatographie en phase gazeuse des constituants glucidiques a été appliquée. La méthode d'extraction par centrifugation permet de recueillir des quantités de solution plus abondantes (4 à 6 fois) que par pipetage (Tabl. 1) ; toutefois la totalité de la fraction glucidique représentative des nectars est intégralement présente dans des solutions prélevées par pipetage (Tabl. 5) : les sucres sont donc dilués par centrifugation. La présence de glucose et de fructose, en proportions voisines, a été mise en évidence (Tabl. 2, 3, 4). L'adoption de la technique de prélèvement par pipetage, qui assure une restitution plus fidèle des sécrétions disponibles pour les insectes pollinisateurs, est préconisée et pourrait permettre la sélection de lignées en vue d'une optimisation de la pollinisation croisée.

ZUSAMMENFASSUNG

UNTERSUCHUNGEN ÜBER DIE BLÜTENNEKTARSEKRETION VON RAPS (*BRASSICA NAPUS* L. VAR. *OLEIFERA* METZGER)

Raps (*Brassica napus* L. var. *oleifera* METZGER), wird zum größten Teil vom Wind bestäubt. Jedoch hängt vor allem die Produktion von F1 Hybrid-Samen von der Aktivität bestäubender Insekten ab. Hierbei spielt die Honigbiene die größte Rolle. Die Nektar sammelnden Honigbienen zeigen eine Präferenz für männlich-fertile Linien, was dadurch zustande kommen könnte, daß Unterschiede in der Nektarsekretion bestehen. Um die im Laufe der Selektion entstandenen Unterschiede in der Nektarsekretion mit einer verlässlichen Methode zu schätzen, wurden zwei Extraktionsmethoden, die Zentrifugation und die Pipettierung (anhand des Nektars der Varietät Kid) verglichen. Über die quantitative Messung der Nektarproduktion hinaus wurde eine gaschromatographische Analyse der Kohlehydrat-Komponenten durchgeführt.

Die Methode der Zentrifugation führt zu einer größeren Ausbeute (4 bis 6 mal) als die Pipettierung (Tab. 1). Dennoch war die Gesamtheit der repräsentativen Kohlenhydrat-Fraktion auch in den pipettierten Proben vorhanden, was darauf schließen läßt, daß in den zentrifugierten Proben die Zucker nur mehr verdünnt sind. Es konnte gezeigt werden, daß Glukose und Fruktose in ähnlichen Proportionen vorliegen (Tab. 2, 3 u. 4).

Die Methode des Pipettierens eignet sich besser, wenn man analysieren will, welche Sekretionsmenge dem bestäubenden Insekt effektiv zur Verfügung steht. Außerdem könnte sie die Selektion von Linien im Hinblick auf eine Optimierung der Kreuzbestäubung begünstigen.

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