FLORAL NECTAR SECRETION OF RAPESEED MALE STERILE CYBRIDS

M.H. Pham-Delegue (1), J. Mesquida (2), R. Marilleau (1), M. Le Métayer (1), J. Renard (3)

(1) Laboratoire de Neurobiologie Comparée des Invertébrés INRA-CNRS (URA 1190), BP 23, 91440 Bures-sur-Yvette, France.
(2) Laboratoire de Zoologie, Centre de Recherches de Rennes, Domaine de la Motte au Vicomte, BP 29, 35650 Le Rheu, France.
(3) Station d'Amélioration des Plantes, Centre de Recherches de Rennes, Domaine de la Motte au Vicomte, BP 29, 35650 Le Rheu, France.

INTRODUCTION

Owing of the dominant autogamy of the rapeseed, the male sterility is necessary to create hybrid varieties. Male sterile cybrids (radish type cytoplasmic male sterility (OGURA 1968), improved by protoplast fusion (PELLETIER et al. 1983)) were obtained. These cybrids need to be cross-pollinated by male fertile plants providing pollen carried by insect vectors, mainly honey bees. A good cross-pollination depends on the attractiveness of both genotypes related mostly to the quality and quantity of their nectar secretion. Honey bees' frequentation is known to be weak on rapeseed genotypes providing few amounts of nectar (MESQUIDA and RENARD 1978), and the sugar quality of the nectars was shown to interfere on bees preferences in sunflower genotypes (FONTA et al. 1985). The influence of such parameters was therefore analyzed in rapeseed male sterile cybrids in F1 hybrid seed production, according to the genotype, the day and the time of nectar collection. The sugar composition of nectar was studied using a high performance liquid chromatography (HPLC) method previously applied to the analysis of floral nectars (ERICKSON et al. 1979).

MATERIALS AND METHODS

Plant material : Five male sterile cybrids of winter rapeseed Darmor : 27, 58, 77, 85, 118 and the male fertile Darmor were studied.

Nectar collection : pipette (5 µl volume) collection of nectar samples was undergone on 5 plants per genotype (2 to 5 flowers per plant). Quantities secreted were directly red on the pipette, before being kept at - 20° C for further analyses. The influence of the day of collection (April 15th, May 3rd) and of the time of the day (8 a. m., 2 p. m. on April 21st) were studied.

Sugar composition : analyses were conducted using an HPLC method. Constant volumes (20 µl) of a reference solution (glucose, fructose, sucrose) and equal volumes of the nectar samples were injected. The concentration of each sugar, expressed in mg of sugar per 100 mg of nectar, was obtained following a calculation method previously described (BLACK and BAGLEY 1978 ; VEAR et al. 1990). Refering to BOLTEN et al. (1979) the data were transformed in mg of sugar per 100 µl of nectar. The values were compared using an ANOVA test with fixed effects model including 3 factors : genotype (27, 58, 85, Darmor ; nectar amounts of 77 and 118 being weak, these values were excluded from the analyses), day or time of collection and blocks. The mean values were then classified using a NEWMAN-KEULS test. The values expressed as percentages were compared after angular transformation (Arcsinus $\sqrt{P}$).

RESULTS

Effect of time collection : nectar secretions were more abundant at 8 a. m. than at 2 p. m. (60 % more, Fig. 1A, FN). The secretions of Darmor were more abundant than those of the cybrids (94 % to 121 % more, Fig. 1B,

FN). Secretions of 118 and 77 were weak (less than 1 % of Darmor in 77, and less than 0.5 to 10 % of Darmor in 118). The weaker secretions collected at 2 p. m. were more concentrated in total amounts of sugar than those of 8 a. m. (184 % more on average, Fig. 1A, DM). The increase was the highest in the genotypes 58 and Darmor (205 and 270 % respectively). Glucose and fructose were identified, as well as traces of sucrose (0.4 to 4.3 %). The mean concentrations of the main sugars were similar and close to 50 % at the 2 times of collection (Fig. 1A, FR and GL).

Effect of the day of collection : significant day and genotype effects appeared (Fig. 2A and B, FN). Amounts of nectar were higher on May 3rd than on April 15th (23 % more). The day effect was particularly strong in genotype 58 (125 % increase). The genotype effect mainly relied on the weaker secretions of the cybrids compared to Darmor (65 to 95 % more in Darmor) (Fig. 2B, FN). The total amounts of sugar were similar in cybrids and Darmor for both days of collection (19.3 to 22.4 mg/100 µl, Fig. 2A and B, DM). The mean concentrations of each sugar were similar and close to 50 % (Fig. 2A, FR and GL).

DISCUSSION

The data obtained from HPLC analysis were similar to those obtained previously from gas chromatography method on rapeseed nectars (MESQUIDA et al. 1988 a). The presence of two main sugars, glucose and fructose, as well as traces of sucrose fit with other works on rapeseed nectars (WYKES 1952 ; PERCIVAL 1961). Therefore HPLC method, which allows to analyses rapidly numerous samples even of small quantities (1 µl), without prior treatment, appears to be well adapted to the sugar caracterisation of floral nectars.

Nectar secretions were shown to vary quantitatively and qualitatively according to genotypes, time and day of collection. Cybrid secretions were always lower than those of Darmor, and differences appeared among the cybrids. These differences may rely on genetic differences, since 77 and 118 were of OGURA type, which cytoplasm was reported to induce low nectar secretion (MESQUIDA and RENARD 1978), and 27, 58, 85 were of rapeseed type, corresponding to abundant nectar secretion (MESQUIDA et al. 1988 b). The total amount of sugar was equivalent in cybrids and in Darmor, as well as the glucidic composition (50 % of fructose and 50 % of glucose).

The reversed variation of quantities secreted and of dry matter concentrations between the morning and the afternoon samplings was previously mentionned (KLEBER 1935 ; FAHN 1949 ; NUNEZ 1973). This may be due to an evaporation process linked to the increasing temperature, relative humidity and sunlight in the afternoon (CORBET et al. 1979). Although the nectar quantities and the amounts of dry matter remained rather constant according to the day of collection, the relative decrease of secretion observed in Darmor between the beginning and the end of the flowering period could rely on an ageing process already mentionned by WILLIAMS (1980).

Eventhough the cybrid secretions may vary according to the pedoclimatic conditions, their glucidic corposition remains stable and representative of the plant species, with amounts of fructose and glucose allowing to expect good pollinators frequentation and consequent use of male sterile cybrid in the production of F1 hybrid seeds.

REFERENCES

BLACK, L. T. and BAGLEY, E. B. 1978. Determination of oligosaccharides in soybeans by high pressure liquid chromatography using an internal standard. J. Ann. Oil Chemists' Soc. 55 : 228-232.

CORBET, S. A., WILLMER, P. G., BEAMENT, J. W. L;, UNWIN, D. M. and PRYS-JONES, O. E. 1979. Post-secretory determinants of sugar concentration in nectar. Plant cell and Environ. 2 : 293-308.

ERICKSON, E. H., THORP, R. W., BRIGGS, D. L., ESTES, J. R., DAUN, R. J., MARKS, M. and SCHROEDER, C. H. 1979. Characterization of floral nectars by high-performance liquid chromatography. J. Apic. Res. 18 : 148-152.

FAHN, A. 1949. Studies in the ecology of nectar secretion. Palest. J. Bot. Jerusalem 4 : 207-224.

FONTA C., PHAM-DELEGUE M. H., MARILLEAU R. and 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 Oecologica, Oecol. Applic. 6 : 165-175.

KLEBER E. 1935. Hat das Zeitgedächtnis der Bienen Biologische Bedentung. Z. vergl. Physiol. 22 : 221-262.

MESQUIDA J. and RENARD M. 1978. Entomophilous pollination of male sterile strains of winter rapeseed (Brassica napus L. var. oleifera) and a preliminary study of alternating devices. Proc. 4th int. Symp. on Pollination, Versailles. pp. 25-37.

MESQUIDA, J., MARILLEAU, R., PHAM-DELEGUE, M. H. and RENARD, M. 1988 a. A study of rapeseed (Brassica napus L. var. oleifera METZGER) flower nectar secretions. Apidologie 19 : 307-318.

MESQUIDA, J., RENARD, M., PELLAN-DELOURME, R., PELLETIER, G. and MORICE, J. 1988 b. Influence des sécrétions nectarifères des lignées mâles-stériles pour la production de semences hybrides F1 de colza. In : Variabilité génétique cytoplasmique et stérilité mâle cytoplasmique. Les Colloques de l'INRA (ed.), Sainte-Sabine, France. 45, pp. 269-280.

NUNEZ, J. A. 1973. Le comportement de butinage de l'abeille (Apis mellifera L.). Etude quantitative du comportement. C. R. Congr. int. d'Apiculture de l'Apimondia, buenos-Aires. pp. 285-290.

OGURA, H. 1968. Studies of the new male sterility in Japanese radish, with special references to the utilization of this sterility towards the practical raising of hybrid seeds. Mem. Fac. Agric. Kagoshima Univ. 6 : 39-78.

PELLETIER, G., PRIMARD C., VEDEL F., REMY R., ROUSSELLE P. and RENARD M. 1983. Intergeneric cytoplasmic hybrizidation in Cruciferae by protoplast fusion. Mol. Gen. Genet. 191 : 244-250.

PERCIVAL, M. S. 1961. Types of nectar in Angiosperms. New Phytol. 60 : 235-281.

VEAR, F., PHAM-DELEGUE, M. H., TOURVIEILLE de LABROUHE, D., MARILLEAU, R., LOUBLIER, Y., LE METAYER, M., DOUAULT, Ph. and PHILLIPON, J. P. 1990. Genetical studies of nectar and pollen production in sunflower. Agronomie 10 : 219-231.

WILLIAMS, I. H. 1980. Oil-seed rape and beekeeping, particularly in britain. Bee World 4 : 141-150.

WYKES, G. R. 1952. An investigation of the sugars present in the nectar of flowers of various species. New Phytol. 51 : 210-215.

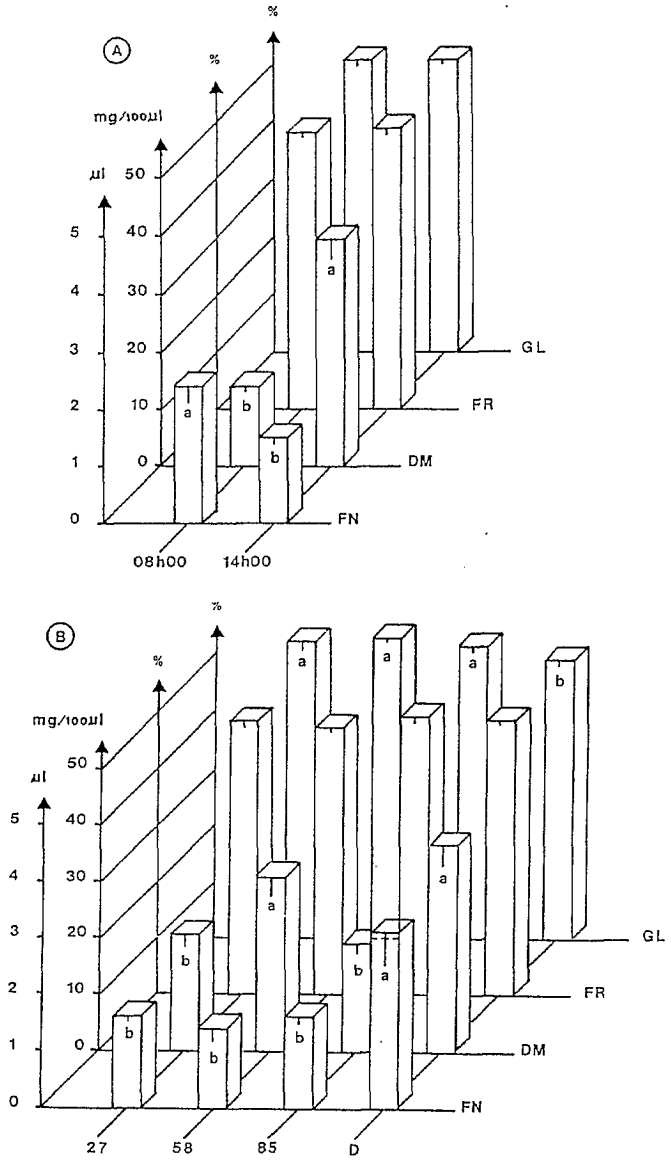

Fig. 1. Mean values of nectar secretion per flower (FN), of dry matter (DM) and of relative concentrations of fructose (FR) and glucose (GL), according to time of collection (A) and genotypes (B) (values affected with different letters are significantly different).

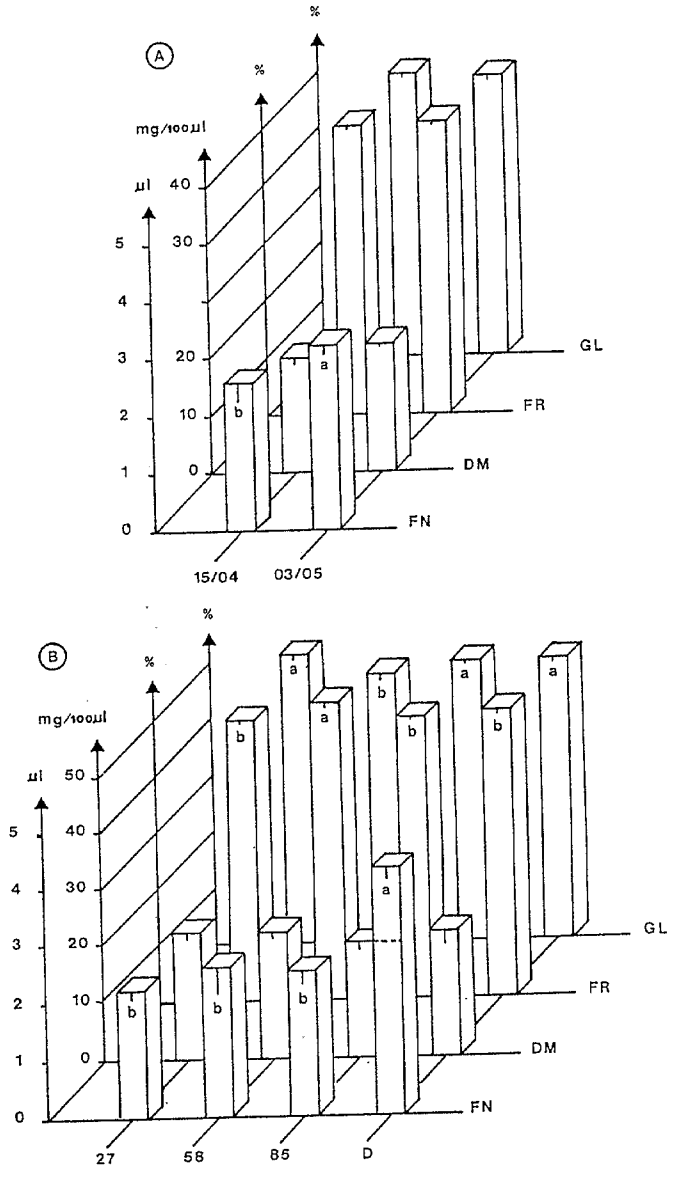

Fig. 2. Mean values of nectar secretion per flower (FN), of dry matter (DM) and of relative concentrations of fructose (FR) and glucose (GL), according to day of collection (A) and genotypes (B) (values affected with different letters are significantly different).