The appropriate technique for collecting and measuring the amount of floral nectar in sunflower (*Helianthus annuus* L.)

Zvonimir Sakac, Sreten Terzic, Vladimir Miklic
Institute of field and vegetable crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia
E-mail: sakacz@ifvcns.ns.ac.yu; terzic@ifvcns.ns.ac.yu; miklicv@ifvcns.ns.ac.yu

ABSTRACT

The available techniques for collecting and measuring the amount of floral nectar are applicable but often found to be unrepresentative. Centrifugation yields larger samples but they also include nectar that is not actually accessible to insects, the capillary method has been described as unsuitable because of possible damage to the nectary tissue, the method including filter paper is considered to be unreliable because of evaporation and nectar extraction methods including washing are considered limited because the solution may include sugars from plant tissue cells. We have found that capillary tubes with inner diameter of 0.25-0.5mm and outer diameter of 0.5-0.75mm are suitable for nectar collection in sunflower. To determine the amount of nectar, we isolate five inflorescences per sunflower line at the start of flowering and collect the nectar two days after the isolation. The capillary tube is inserted between the style and filaments down to the nectary. After the level of nectar stops rising the next flower is processed. The tubes can be measured on an analytical scale and the amount of nectar is obtained as the weight increase in comparison to the empty tube. Faster determination of floral nectar amount can be provided by using calibrated capillary tubes of a known and uniform inner diameter. The appropriate outer diameter of the capillary tubes reduces the risk of tissue damage and allows more precise collecting so that the capillary method is preferable to others for nectar collecting in sunflower.

Key words: capillary technique – nectar quantity – sunflower

INTRODUCTION

Sunflower is one of the plant species that produces pollen which is too heavy for wind dispersal (Putt, 1940). Even though the cultivated sunflower has a reasonable percentage of self-compatibility it still benefits from insect pollination. One of the major components influencing pollinator choice is certainly the production of nectar, whose amount and quality are often studied.

The nectaries in *Asteraceae* family form on top of the ovary and surround the style base (Mani and Saravanan, 1999), (Fig. 1). The nectar can be accessed for quantification purposes by capillary tubes (Hocking, 1953), volumetric centrifugation (Bosi, 1973), filter paper strips (McKenna and Thomson, 1988), flushing of water into the corolla (Cresswell and Galen, 1991) or floating the flowers inverted in water (Manetas, 2000) depending on flower structure. These techniques were used with a variable success, but the overall conclusion is that no single method can be considered satisfactory for all plant species (Mesquida et all, 1988). The capillary method can be used in sunflower but it is necessary to use capillary tubes of appropriate dimensions. If the outer diameter is too large it is not possible to access the nectar without destroying the surrounding corolla tissue (Fig. 1.) and if the tube is too thin then the intake of nectar is slower and the strong capillary force may make the extraction of nectar from the tubes difficult.

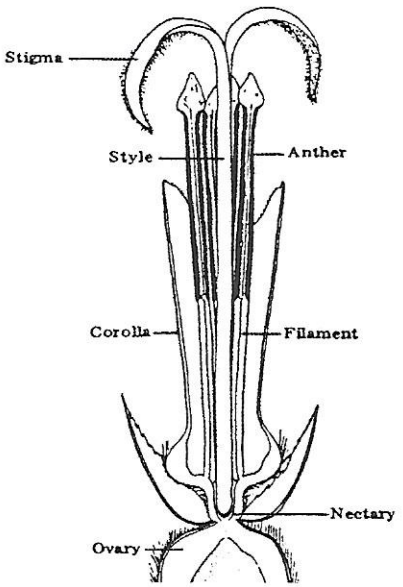

Fig. 1. Longitudinal section of a sunflower disk flower showing the location of the nectary

MATERIALS AND METHODS

We found that capillary tubes with inner diameter of 0.25-0.5mm and outer diameter of 0.5-0.75mm are suitable for nectar collection. The length of the tube then determines the capacity and can be picked for its suitability to the collecting design but should not be smaller than 15 µl.

To determine the amount of nectar, we isolate five inflorescences per sunflower line at the start of flowering with linen bags to prevent insects from collecting nectar. The best moment is when the first two rows of disk flowers have opened. Two days after the isolation, at approximately 8 AM, the inflorescences are cut and taken to the laboratory in a portable refrigerator to minimize evaporation and the change in nectar volume. It is advisable for the transport duration to be as short as possible. The following should be prepared to analyze one inflorescence:

1. Four capillary tubes (previously weighed on an analytical scale) each placed in a separate tube labeled with sample and replication to ease the work of collecting and measuring
2. A clean vegeglass (previously weighed on an analytical scale)
3. A clean HPLC vial with sample label on it filled with 1 ml mixture of AcCN:H_2O in a ratio of 75:25
4. A plastic dish with sample label on it for deep freezing

Four groups of five analyzed flowers are equally far from each other on an inflorescence. We collect nectar from 5 fully opened disk flowers with one non calibrated capillary tube. The tube is inserted between the style and filaments down to the nectary (Fig. 1.). After the level of nectar stops rising the next flower is processed. When a total of 20 flowers are finished, the tubes can be measured on an analytical scale and the amount of nectar is obtained as the weight increase in comparison to the empty tube. Faster determination of nectar amount in flowers can be provided by using calibrated tubes (capillary tubes with a uniform known inner diameter) for nectar collecting, in which case the height of nectar in tubes can be correlated with the nectar volume. This method is suitable when it is necessary to determine the amount of nectar in field conditions, without cutting the sunflower head and taking it to the lab.

The next step in method developing is a qualitative and quantitative HPLC analysis of nectar extracted from a single inflorescence as a collection from 20 disk flowers. For this purpose, ten disk flowers are also pulled off with tweezers, put in a glass and weighed on an analytical scale to obtain the information about the flower mass and possible correlation with nectar production.

The nectar collected in capillary tubes, after weight measurement, can also be kept for subsequent analysis. The contents of all capillary tubes from a single inflorescence are transferred into a HPLC vial (2 ml) filled with 1 ml mixture of AcCN:H_2O in a ratio of 75:25 and placed in a refrigerator for

subsequent HPLC nectar quality analysis. Twenty flowers are pulled out of the disk with tweezers, frozen in liquid nitrogen and then kept at -72°C.

DISCUSSION

The rest of the techniques cited are applicable but often unrepresentative. Centrifugation yields larger quantities but they also include nectar that is not actually accessible to insects and modified chemical composition due to tissue lesion (Mesquida et al., 1988). The method including filter paper is considered to be unreliable because of evaporation (Livtzieva, 1954). Nectar extraction methods including washing are considered limited because the solution may include sugars from plant tissue cells (Kenoyer, 1917).

A combination of capillary method and filter paper can be used so that the nectar is extracted with capillary tubes and than ejected on to a filter paper, which is measured for total nectar. After the evaporation has finished the amount of sugars is obtained as a difference between wet and dry filter paper.

The capillary method has been used on sunflower (Pham-Delegue et al., 1985) but it has also been described as unsuitable for collecting nectar amounts less than 1 µl and to cause damage to the nectary tissue (McKenna, 1988). The appropriate outer diameter of the capillary tubes reduces the risk of tissue damage and allows more precise collecting so that the capillary method is preferable to others for sunflower nectar collecting.

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