Counting pollen grains of some almond cultivars by means of an haemocytometer

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Couting pollen grains of some Almond cultivars by means of an haemocytometer

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RESUME-ABSTRACT

The pollen grain production of ten almond cultivars was evaluated in 1978 and 1979, by means of a "Bürker" haemocytometer. In 1979 the number of pollen grains greatly decreased in all the cultivars, maybe because of the reduced microsporogenesis due to cold spells and frosts on January.
In pollen production cultivans showed significant differenas per anther and per flower: "Ferrante", "Genco", "Scorza verde" and "Texas" were good pollen producers, whereas "Fragiulio grande", "Nonpareil" and "Tuono" were poor producers.

Le nombre de grains de pollen par anthère et par fleur a été évalué, pour 10 variétés d'Amandier, à l'aide d'un hémacytomètre de "Burker" en 1978 et 1979. Ce nombre a été nettement plus faible en 1979 (influences climatiques).
Il existe des différences variétales sensibles: Ferrante, Genco, Scorza verde et Texas étant les plus productives, alors que Fragiulio grande, Nonpareil et Tuono étaient les moins productives.

INTRODUCTION

Cross-pollination is required by self-incompatible almond cultivars to produce a crop, but it is also useful for increasing fruit set of self-compatible ones.[6]

In order to maximize the effects of cross-pollination by honeybees, it becomes necessary that the cultivars: 1) overlap in blooming; 2) produce viable pollen; 3) be intercompatible and 4) arranged in the orchard according to adequate layouts.

In choosing pollinators cultivars, not only the quality, but also the quantity of pollen produced per anther and per flower are to be considered. In fact, under the above conditions, some cultivars could be preferred to others as pollinators because of the more abundant pollen production.[8]

The purpose of the present work was to evaluate the pollen production of 10 almond cultivars, most of Apulian origin, by the aid of the haemocytometer, a tool widely used in the clinical field for counting blood cells.

The earliest attempts to count pollen grains were based upon not accurately defined methods.[3] The first application of the haemocytometer dates back to

1952, when the "Spencer Bright Line" was used, revealing an interesting variety of differences in some fruit species.[8]

In investigations carried out in Italy the "Thoma" haemocytometer was used to count the pollen grains of several fruits dispersed in ethyl alcohol.[2 5 9]

In our studies we used a "Bürker" haemocytometer, more suitable than the "Thoma" total because of its considerably wider counting chambers.

Our methods, adopted by other authors[1 7] allowed them to obtain satisfactory results with cherry and almond.

MATERIALS AND METHODS

The anthers of 10 almond cultivars grown in the experimental orchard at Bari-Palese were collected in 1978 and 1979 from flowers in D stage.[4]

For each cultivar, six samples of 100 anthers were prepared and placed in as many vials. The drying and dehiscence of anthers in vial were obtained first at room temperature, then in a stove at 50 °C for 6 hours. Nevertheless, too many pollen grains were still stuck to the endothecium, so that it proved necessary to free most of the grains by triturating carefully the anthers with a glass rod.

Two ml of 5% acqueous solution of a detergent (Teepol of Shell Chemical) were poured in each vial and a uniform suspension of grains was obtained after shaking.

The chambers of the "Bürker" haemocytometer were filled with drops of the suspension with micropipette.

The "Bürker" haemocytometer is a microscope slide with 2 counting chambers, each of 9 mm^2 surface; a cover slip gives the chambers a depth of 0.1 mm. The inner structure of the chambers is rather complex. However, we just synthetize, in regard to our studies, that each chamber is subdivided in 9 small chambers, each having a surface of 1.00 mm^2, a depth of 0.1 mm and a volume of 0.01 ml.

The counting of pollen grains was made inside the small chambers, each retaining $\frac{1}{20{,}000}$ of the original amount of the 2 ml of the suspension per vial.

The number of pollen grains per anther (A) was calculated as follows: $A = \frac{n \times B.}{N}$

where: n = number of pollen grains counted inside each small chamber;

B = fraction of suspension retained by each small chamber in relation to the original amount of suspension of each vial;

N = number of anthers per vial.

In our case, $A = \frac{n \times 20{,}000}{100} = n \times 200.$

The counts of grains in each of the 9 small chambers were averaged and multiplied x 200. This procedure was repeated six times per vial.

The number of pollen grains per flower was determined multiplying the average number of grains per anther in each vial times the average number of anthers per flower, calculated on samples of fifty flowers per cultivar and year.

On five samples of 200 pollen grains, viability tests were made with a vital stain (Aceto-carmine) in order to ascertain the number of viable grains per anther and per flower.

RESULTS

Anthers of the cultivars contained slightly less than 1,500 pollen grains in average (Table 1).

The use of the "Bürker" haemocytometer has allowed to ascertain a remarkable variety of differences. In particular, the anthers of "Cristomorto", "Genco", "Scorza verde" and "Texas" contained a relatively high number of grains (more than 1,600), whereas anthers of "Fragiullo gr.", "Nonpareil" and "Tuono" contained a low number of grains (less than 1,300).

It is worth pointing out that the pollen production in 1979 was decreased by more than 20% in average with respect to 1978. Such a decrease, more or less equal in all the cultivars, may be ascribed to the se vere climatic conditions of January 1979, when minimun temperatures often fell below 0°C. Temperature drops are likely to have slowed down microsporogenesis, thus reducing formation of pollen grains in the anthers.

The mean number of anthers per flower was 32.6 (Table 2). However, significant differences were observed among cultivars: "Ferrante" and "Texas" had more than 35 anthers per flower, whereas "Cristomorto"

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Table 1
Total number of pollen grains per anther.

Cultivars	Year			Mean[yy]
	1978	1979	Signif.[y]	
Cristomorto	1,811	1,449	++	1,630 AB
Falsa barese	1,757	1,305	++	1,530 AC
Ferrante	1,503	1,287	++	1,395 BD
Filippo Ceo	1,685	1,384	++	1,535 AC
Fragiulio gr.	1,313	885	++	1,099 E
Genco	1,979	1,595	++	1,787 A
Nonpareil	1,433	1,102	++	1,268 CE
Scorza verde	2,046	1,470	++	1,758 A
Texas	1,826	1,442	++	1,634 AB
Tuono	1,364	992	++	1,178
Mean	1,672	1,291	++	1,482

y — ++=significant at 0.01 P.
yy — Different letters mark values significant at 0.01 P.

and "Filippo Ceo" no more or less than 30 anthers per flower.

In both years, no correlation has been observed between the number of anthers per flower and the number of pollen grains per anther.

Obviously, the different number of anthers per flower influenced the total number of pollen grains per flower, thus at least modifying partially the previous ranking. In particular, cultivars producing more than 50,000 grains per flower were "Falsa barese", "Ferrante", "Genco", "Scorza verde" and "Texas"; those producing less than 41,000 grains were "Fragiulio gr.", "Nonpareil" and "Tuono".

Viability of pollen was very high in all the cultivars, averaging 97,2%. However, significant differences were also found among cultivars. Taking pollen viability into consideration did not change the ranking of cultivars, except for "Falsa barese", with respect to production of viable pollen grains per anther and per flower (Table 3).

CONCLUSIONS

"Bürker" haemocytometer proved to be an extremely useful tool for carrying out easy and precise counts of pollen grains per anther.

The present study concerning almond as well, con-

Table 2
Mean number of anthers and of pollen grains per flower.

Cultivars	Anthers (No)	Pollen Grains (No)
Cristomorto	20.4 E	46,445 BC
Falsa barese	33.4 B	51,073 AB
Ferrante	37.2 A	52,044 AB
Filippo Ceo	30.1 DE	46,271 BC
Fragiulio gr.	33.1 BC	36,960 C
Genco	31.8 BD	56,776 A
Nonpareil	32.8 BD	40,644 C
Scorza verde	30.8 CD	54,434 AB
Texas	36.0 A	58,986 A
Tuono	33.4 B	39,962 C
Mean	32.6	48,360

— Different letters mark values significant at 0.01 P.

Table 3
Mean number of viable pollen grains per anther and per flower

Cultivars	Viability (%)	Anther (N.°)	Pollen grains per flower (N.°)
Cristomorto	96.7 BD	1,576 AB	44,927 CE
Falsa barese	95.1 D	1,455 AC	48,574 BD
Ferrante	97.6 AC	1,361 BD	50,773 AC
Filippo Ceo	97.9 AC	1,504 AC	45,360 CE
Fragiulio gr.	97.6 AC	1,071 E	36,009 E
Genco	96.7 BD	1,731 A	54,985 AB
Nonpareil	98.4 AB	1,247 CE	39,997 DE
Scorza verde	96.9 BC	1,702	52,705 AC
Texas	99.1 A	1,628 AB	58,785 A
Tuono	96.5 CD	1,135 DE	38,484 E
Mean	97.2	1,441	47,060

— Different letters mark values significant at 0.01 P.

firms the existing differences related to cultivars and their yearly periodical observations; the latter due to climatic variations.[8]

The data obtained, if related to bloom density, could provide a better insight on the pollen-producing behavior of cultivars.

Based upon the results of this preliminary study, it seems possible to conclude that out of the 10 investigated cultivars, only "Ferrante", "Genco", "Scorza verde" and "Texas" were good producers of viable pollen, whereas "Fragiulio gr", "Nonpareil" and "Tuono" were comparatively less satisfactory producers.

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