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Observing pollen tube growth in self-compatible Almond cultivars by means of fluorescence

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RESUME-ABSTRACT

The use of fluorescence microscopy, in order to observe tube pollen growth into styles, allowed to visualize clearly the overcoming of incompatibility barrier in selfed styles of cv "Filippo Ceo" and "Genco", but also to point out a faster growth in those cross-pollinated with "Tuono" pollen.

La pollen a commencé à germer après 48 heures sur les stigmates dans nos conditions d'expérience. La croissance de l'auto-pollen a démontré à nouveau que GENCO et FILIPPO CEO étaient autocompatibles. Cependant, le pollen se développe plus rapidement dans le cas de pollinisation croisée avec Tuono: 90 et 70 p. 100 des styles montrent des tubes polliniques ayant atteint leur base respectivement chez FILIPPO CEO et GENCO, contre 40 et 30 p. 100 dans le cas de l'autofécondation.

INTRODUCTION

To date, eight almond cultivars, all of Apulian origin, are known to be self-compatible, as a consequence of natural self-pollination of flowers bagged during the blooming period.^{2 6 8} Evidently, in these cultivars pollen tubes penetrate selfed styles fast enough to reach and fertilize oospheres before their receptiveness declined.

However, fruit set of naturally selfed flowers of these cultivars was generally lower than that of hand cross-pollinated ones,⁴ probably because of faster growth of pollen tubes after cross-pollination than after selfing,¹² or of higher efficiency of hand-pollination, in comparison with natural pollination.

All the authors who investigated pollen tube growth in

flowers of almond and almond-hybrids^{7 12 13} used Lactoid stain, and pointed out that, in the orchard, the complete penetration of cross-pollinated styles, depending on genotype and temperature, does not occur before 96 hours from pollination.

In the present paper we report the results of studies carried out using fluorescence microscopy on two self-compatible almond cultivars, in order to visualize the overcoming of incompatibility barriers and to examine and compare the rapidity of style penetration by pollen after self- and cross-hand-pollination.

MATERIALS AND METHODS

Investigations were carried out in February 1977, in the experimental orchard of Bari-Palese, on adult trees of cv "Filippo Ceo" and "Genco".

Table 1
Weather conditions

	FEBRUARY 1977								
	20	21	22	23	24	25	26	27	28
TEMP. (°C)									
Min.	11	12	14	14	11	10	10	10	6
Max.	18	19	22	23	21	21	16	15	12
Mean	14.3	14.7	16.8	16.5	15.1	16.0	13.0	12.2	9.4
Hrs. > 20 °C (no)	=	=	4	6	4	3	=	=	=
Hrs. < 10 °C (no)	=	=	=	=	=	=	=	=	8
Rainfall (mm)	=	=	=	=	=	=	=	=	3.8

At full bloom (Feb. 19 and 22 for "F. ceo" and "Genco" respectively) flowers in D stage¹ were emasculated; at the same dates, samples from anthers of both cultivars and of cv "Tuono", were collected and allowed to dry and dehisce at room temperature (20-22 °C).

About 24 hours after emasculation, half of the flowers of each cultivar were selfed and half were cross-pollinated with "Tuono" pollen.

For each cultivar and each treatment, samples of 15 pistils were collected at 0, 24, 48, 72, 96, 120 hours after pollination, fixed immediately in 95 % ethyl alcohol and glacial acetic acid (3:1 v/v) and stored at 3-5 °C.

The lengthening of pistils was measured and the presence of stigma browning, as a sign of a decline in receptiveness, was observed.

Attempts made in 1977 using Lacmoid stain proved unsatisfactory. Thus, fluorescence microscopy was adopted throughout 1978 for observing pollen tube growth.

The styles were separated from ovaries, washed in tap water for 2 hours and macerated in 8N Na OH for 6 hours. After being rinsed in water for 1 hour, the material was placed overnight in a 0.1 % aniline blue (w.s.) in 0.1 M K₃PO₄ solution.⁹ Next morning, the styles were placed in a drop of the staining solution on a microscope slide, and squashed carefully under a cover slip and observed with the aid of a HBO 200W high pressure Hg lamp under a Zeiss photomicroscope III.

The length of pollen tubes was measured on samples of 10 styles for each cultivar, each treatment, and each time, and it was reported as the percentage of style length penetrated by the longest pollen tube.

Meteorological data were recorded from a weather station situated near the orchard.

RESULTS

The weather was undoubtedly favourable for pollen tube growth⁷ during the period indicated in Table N.° 1.

No rainfall occurred, except on Feb. 28, and temperatures ranged very often around high values, minimum being always above 10 °C, except on Feb. 28, and maximum above 20 °C four days out of nine, notwithstanding under cloudy or overcast sky.

Pollinations on Feb. 20th and 23rd were with temperatures ranging between 16-17 °C and 20-22 °C, respectively.

With respect to pistil lengthening, the cultivars behaved differently (Table N.° 2): pistils of "F. Ceo" grew continuously up to 96 hours from pollination, whereas those of "Genco" ceased to grow at 72 hours. At the beginning, pistils of both cultivars were of the same length; moreover, at 120 hours, those of "F. Ceo" much longer (max. increase 70,8 %) than those of "Genco" (max. increase 33,5%).

Traces of stigma browning did not become apparent up to 96 hours from pollination. Also in this respect,

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Table 2
Pistil lengthening and stigma browning at different times after pollination.

Cultivar	Hours after pollination					
	0	24	48	72	96	120
	A) Pistil lengthening (mm)					
Filippo Ceo	12.8f	16.1de	18.2c	19.5b	20.8a	21.8a
Genco	12.8f	15.4e	15.6e	16.4de	17.1d	17.2d
	B) Stigma browning (%)					
Filippo Ceo	0.0	0.0	0.0	0.0	5.0c	40.0b
Genco	0.0	0.0	0.0	0.0	25.0b	70.0a

— Different letters mark values significant at 0.01P.

the cultivars behaved differently: in "F. Ceo", browned stigmas increased from 5% to 40% between 96 and 120 hours; in "Genco", at the same periods, browned stigmas were 25% and 70%, respectively.

Observation by means of fluorescence did not show evidence of any effective tube growth during the first 24 hours from pollination (Table N.º 3; Figures N.º 1, 2, 3). Tube elongation was detected only from 48 hours onward, in both cultivars and treatments.

As from 48 hours, "Tuono" pollen tubes grew faster than selfed ones. At 120 hours, penetration of "F. Ceo" and "Genco" pollen tubes into own styles averaged 85.2% and 82.5% whereas penetration of "Tuono" pollen tubes averaged 99.8% and 97.6% of "F. Ceo" and of "Genco" styles, respectively.

In particular, "Tuono" pollen tubes reached the basis of 50% and 90% of "F. Ceo" styles and the basis of 20% and 70% of "Genco" styles after 96 and 120 hours. At the same times, complete penetration of selfed styles interested only 10% and 40% of "F. Ceo" styles, only 10% and 30% of "Genco" styles (Table N.º 4).

No selfed styles of both cultivars showed swollen tubes, the typical symptom of incompatibility.

DISCUSSION

Pistil lengthening continued markedly during anthesis, and, perhaps, it was enhanced by pollination. However, pistils of "Genco" appeared to be less durably

receptive, as indicated by both the earlier stop in elongation and appearance of stigma browning. We do not believe that the slightly lower temperatures under which "Genco" bloomed had a significant influence on the behaviour of its pistils. Considering the shortness of blooming of "Genco",⁵ the effective period for a useful pollination and fertilization of single flowers of this cultivar is limited, as compared with that of other cultivars as, for instance, "F. Ceo".

In cultivars and in effective tubes, independently from pollen source and temperature influence, growth was only observed 48 hours after pollination, as a possible consequence of the time required by proteins of pollen *intine* for stigma recognition.^{10 11} In this connection, our results do not tally with those of other authors,⁷ who observed faster initial tube growth in the orchard, under lower temperature levels, even on different cultivars.

Visualization of pollen tube growth into selfed styles confirmed, beyond doubt, the self-compatibility of the two cultivars and their possibility to fruit set by self-pollination.

However, cross-pollination resulted more efficient than self-pollination, due to the higher percentage of styles of "F. Ceo" and "Genco" completely traversed in 4÷5 days by "Tuono" pollen.

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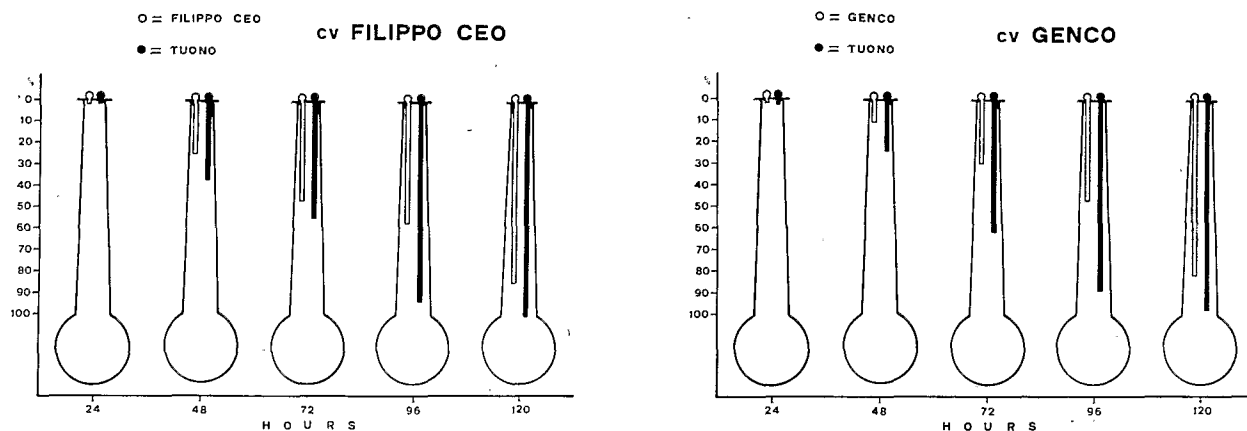


Figure 1. — Mean of the percentages of styles of cvs "Filippo Ceo" and "Genco" penetrated by the longest pollen tube after self-(white) and cross- (black) pollination at different times.

Table 3
Mean of the percentages of style length penetrated by the longest pollen tube after self and cross-pollination at different times.

Cultivar	Pollen	Hours after pollination				
		24	48	72	96	120
Filippo Ceo	selfed	0.9g	25.1f	47.4e	56.9d	95.2b
	"Tuono"	0.7g	37.7e	55.0c	94.6b	99.8a
Genco	selfed	0.9f	11.0e	29.4d	46.8c	85.2b
	"Tuono"	1.1f	24.1d	62.3c	89.2b	97.6a

— different letters mark values significant at 0.01P.

Table 4
Mean of the percentages of style completely penetrated by pollen tubes after self [and cross] pollination at different times.

Cultivar	Pollen	Hours after pollination				
		24	48	72	96	120
Filippo Ceo	selfed	0.0	0.0	0.0	10.0c	40.0b
	"Tuono"	0.0	0.0	0.0	50.0b	90.0a
Genco	selfed	0.0	0.0	0.0	10.0c	30.0b
	"Tuono"	0.0	0.0	0.0	20.0bc	70.0a

— different letters mark values significant at 0.01P.

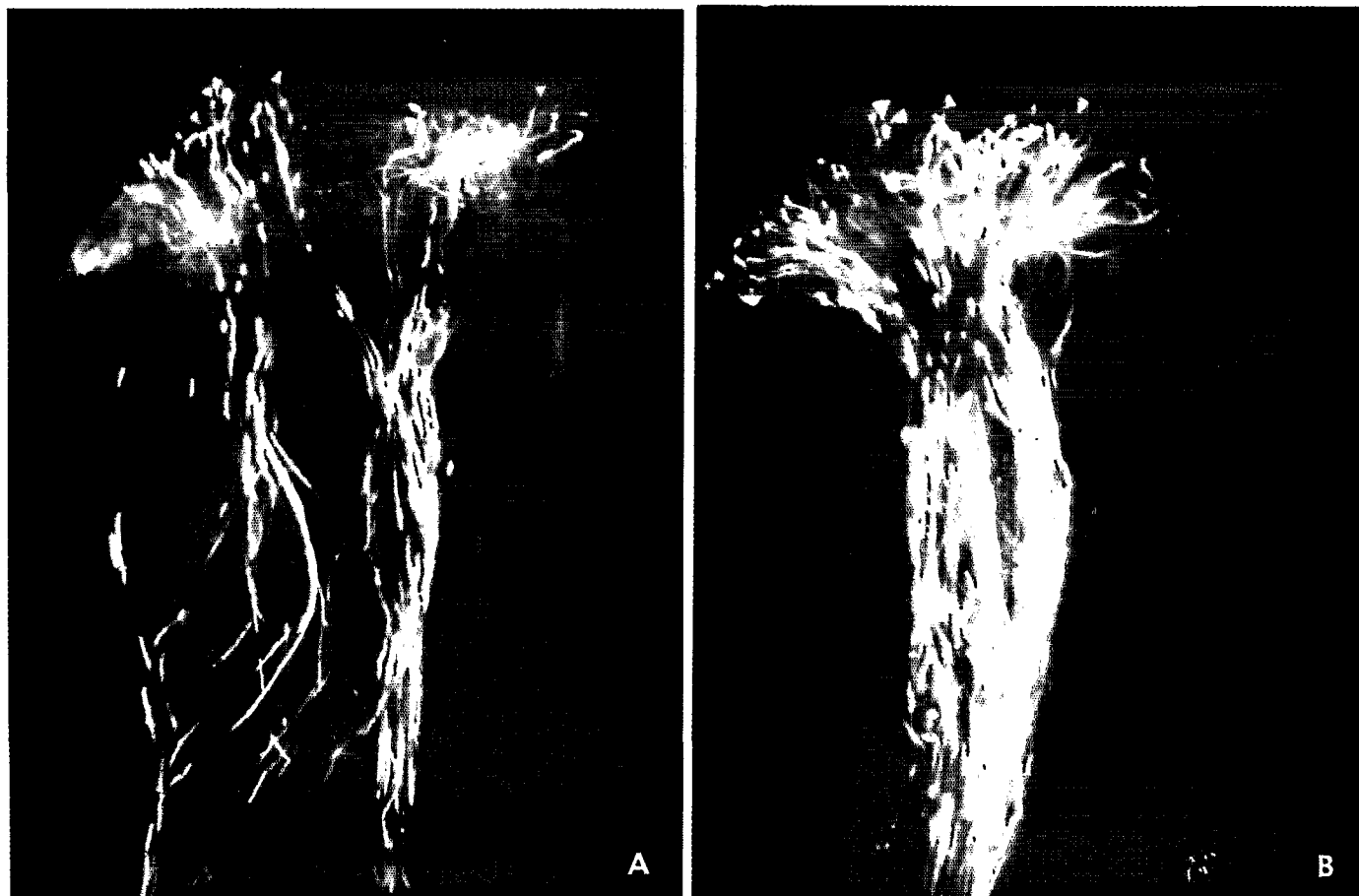


Figure 2. — Pollen tube growth into “Filippo Ceo” styles after 72 hours from self-pollination (A) and from crosspollination (B) with “Tuono” pollen.

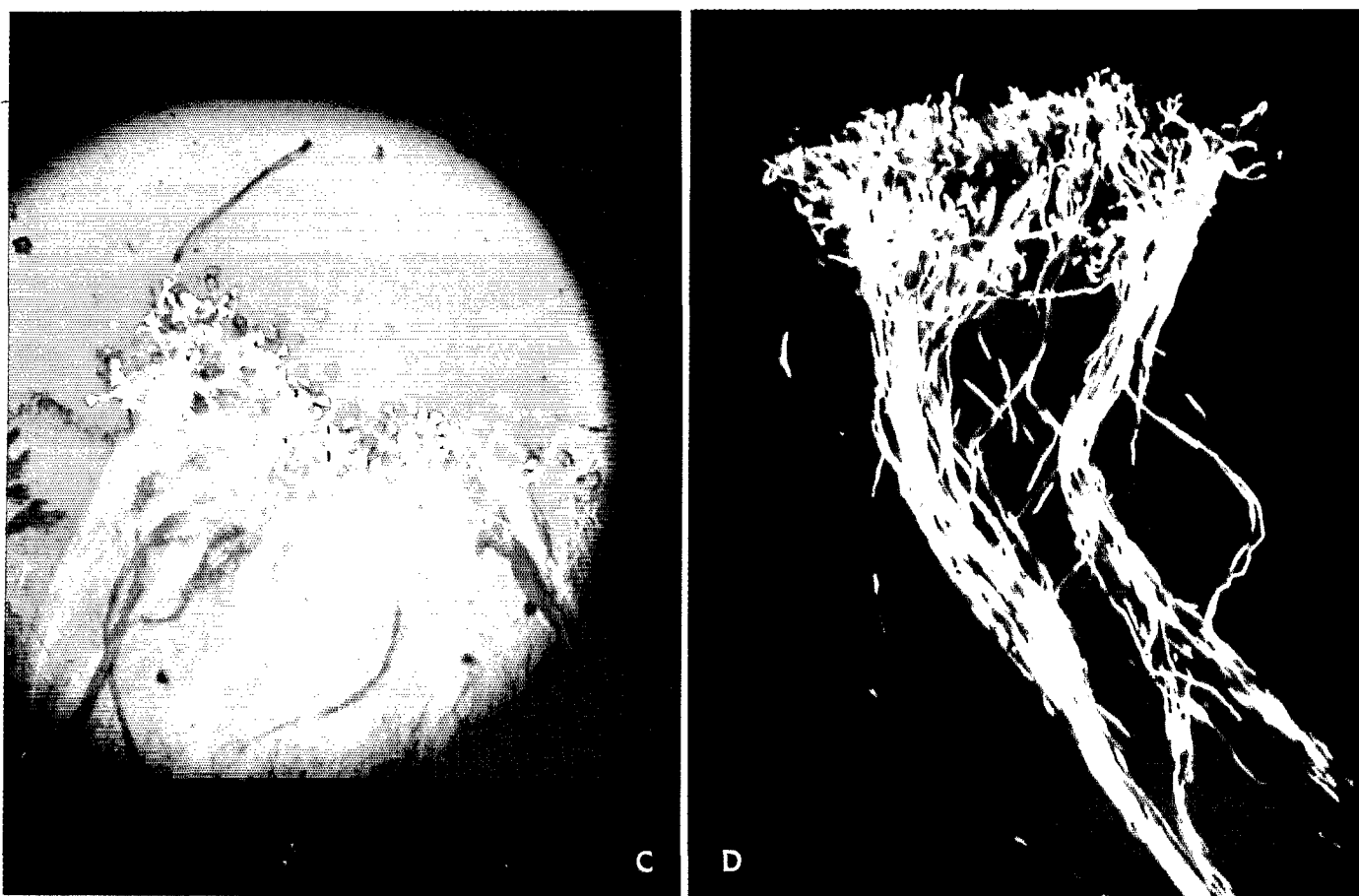


Figure 3. — Pollen tube growth into “Genco” styles after 24 (C) and 72 (D) hours from cross-pollination with “Tuono”.

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