

## REDUCED POLLEN TUBE GROWTH AND THE PRESENCE OF CALLOSE IN THE PISTIL OF THE MALE FLORAL STAGE OF THE AVOCADO

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(Received 25 January 1977)

### ABSTRACT

Sedgley, M., 1977. Reduced pollen tube growth and the presence of callose in the pistil of the male floral stage of the avocado. *Scientia Hort.*, 7: 27—36.

Pollen tube growth was studied in the pistil of the male floral stage of the avocado. Self-pollination of the male stage occurred but pollen tubes did not reach the ovary. When female and male stages were hand pollinated, fewer pollen tubes were present in the pistil of the male than in the female stage. A tube reached the ovary in only 1 of 110 male stage pistils, as compared with all female stage pistils observed. Flowers pollinated in the female stage were retained on the plant for longer than flowers pollinated in the male stage.

Aniline blue-positive and resorcin blue-positive material (callose) was not present in the pistil of flowers in the female stage but occurred in 46 % of the aniline blue-stained and 30 % of the resorcin blue-stained pistils in the male floral stage. By 42 h after first opening, callose was present in every pistil observed, in association with the cell walls of the stigmatic papillae, the transmitting tissue, the vascular tissue or the cortex and epidermis or a combination of these tissues. Callose occurred earlier in pollinated than in unpollinated pistils, particularly in the transmitting tissue.

It is suggested that reduced pollen tube growth in the pistil of the male floral stage of the avocado is associated with the presence of callose.

### INTRODUCTION

Avocado (*Persea americana* Mill.) flowers show marked dichogamy (Robinson and Savage, 1926) and various authors have reported that pollination of the male floral stage does not result in fruit development (Bringhurst, 1952; Peterson, 1956). However, Schroeder (1954) observed that pollen, transferred by direct contact due to the close proximity of the anthers to the stigma in the male floral stage, would germinate and penetrate the stigma. The present study was undertaken to investigate further the behaviour of the pollen tube in the pistil of the male floral stage of the avocado and subsequent fruit retention.

## MATERIALS AND METHODS

Two- and 3-year-old grafted avocado plants, cultivar 'Fuerte', which had initiated flower buds were placed in growth cabinets maintained at 25°C during the day and 20°C at night, with a 12-hour photoperiod and light intensity of 26,000 lux.

The floral cycle under these conditions has been described by Sedgley (1977). The flowers first opened in the afternoon and were in the female stage (0 h). After closing overnight they re-opened in the male stage the next morning (18 h). Closure of the male stage was in progress by 24 h, and the flowers then remained closed. All flowers were labelled at 0 h.

*Pollen tube growth* — (a) Self pollination: Flowers either received no treatment or were emasculated at 0 h. Pistils were collected at 24 h.

(b) Hand pollination: Flowers were pollinated either at 0 h with pollen which had been collected from flowers in the male stage and stored desiccated at 4°C for 6 h (Schroeder, 1942), or at 18 h with fresh pollen. All pistils were collected 4 h after pollination.

Pistils were fixed in Carnoy's fluid at 25°C for 18 h, hydrated and softened for 1 h at 60°C in 0.8 N sodium hydroxide. They were stained with a 0.1 % solution of water soluble aniline blue (George T. Gurr Ltd. Lot No.06604) in 0.1 N tripotassium orthophosphate for 18 h (Martin, 1959), and mounted in 80 % glycerol. Preparations were observed using a Zeiss photomicroscope II with reflected light from an HBO 100 super pressure mercury lamp, BG 3 exciter filter and 65/50 barrier filter combination. Pollen tubes were counted as described by Sedgley (1976).

*Flower retention.* — Flowers were either left unpollinated, pollinated at 0 h or pollinated at 18 h. Each flower was observed daily and time to abscission noted. The average flower retention time after the 3 treatments was calculated.

*Callose localisation* — Flowers were either pollinated at 0 h with stored pollen or left unpollinated. Pistils were collected at 0, 18, 24 and 42 h and fixed either in Carnoy's fluid or 3 % glutaraldehyde in 0.025 M phosphate buffer pH 7.0, both at 25°C. Aniline blue-stained squashes were prepared as described previously and 50 pistils were collected for each treatment and time period for fixation in Carnoy's fluid, and 6 for each treatment and time period for fixation in glutaraldehyde. A further 12 unpollinated pistils were fixed at 42 h in glutaraldehyde at 25°C: frozen 10- $\mu$ m thick longitudinal sections were obtained from 10 pistils using a cryostat and the other 2 pistils were embedded in glycol methacrylate according to the method of Feder and O'Brien (1968). The glycol methacrylate was removed from 2.25- $\mu$ m thick longitudinal sections with acetone using the method of Bencosme et al. (1959). Sections were mounted in a 1 : 1 mixture of aniline blue stain and 80 % glycerol in 0.2 M phosphate buffer pH 8.0. Preparations were observed in ultra violet light as described

previously. For each treatment, time period and method of preparation involving aniline blue staining, a control specimen received 0.1 N tripotassium orthophosphate without the stain.

A further 10 pistils per treatment per time period were processed as for aniline blue-squash preparations but were stained for 18 h with 1.5 % resorcin blue (George T. Gurr Ltd. Lot No.04140) in 50 % ethanol (Reynolds and Dashek, 1976). Preparations were observed using bright field illumination.

## RESULTS

*Pollen tube growth.* — Pollen tubes were not visible in the pistil of the avocado unless stained with aniline blue or with resorcin blue.

(a) Self pollination: No pollen was present in the emasculated pistils (Table I). Small numbers of pollen tubes were present in the stigma and style, but none in the ovary of the untreated flowers.

(b) Hand pollination: Fewer pollen tubes were present in the pistils of the flowers pollinated at 18 h than in those pollinated at 0 h (Table 2). A pollen tube reached the ovary in only 1 pistil pollinated at 18 h as compared with every pistil pollinated at 0 h.

TABLE 1

Pollen tubes counted in pistils of self-pollinated and emasculated avocado flowers at 24 h

	Stigma	Style	Style	Style and ovary
Distance from surface of stigma (mm)	0—0.5	0.5—1.5	1.5—2.5	2.5—3.5
Average no. of tubes per self-pollinated pistil (110 observations)	5.00 ± 0.75	1.57 ± 0.27	0.16 ± 0.04	0.00 ± 0.00
Average no. of tubes per emasculated pistil (12 observations)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

*Flower retention.* — Flowers pollinated at 0 h were retained on the plant longer than flowers pollinated at 18 h or unpollinated flowers and there was no difference between the latter 2 treatments (Table 3).

*Callose localisation.* — Callose was not visible in the pistil of the avocado unless stained with aniline blue or with resorcin blue.

TABLE 2

Pollen tubes counted in pistils of avocado flowers hand-pollinated at 0 h (female stage) and 18 h (male stage)

	Stigma	Style	Style	Style and ovary
Distance from surface of stigma (mm)	0-0.5	0.5-1.5	1.5-2.5	2.5-3.5
Average no. of tubes per pistil pollinated at 0 h (20 observations)	71.90 ± 6.35	20.25 ± 2.12	3.05 ± 0.34	1.00 ± 0.00
Average no. of tubes per pistil pollinated at 18 h (110 observations)	45.50 ± 1.95	7.66 ± 0.59	0.51 ± 0.07	0.01 ± 0.01

TABLE 3

Retention of avocado flowers hand-pollinated at 0 h (female stage) at 18 h (male stage) and left unpollinated

Treatment	Total number of flowers observed	Average number of days each flower retained
Pollinated at 0 h	323	9.37 ± 0.38
Pollinated at 18 h	319	3.97 ± 0.24
Unpollinated	357	4.32 ± 0.20

The occurrence of aniline blue-positive material in the component tissue of the pistil at different times after first opening of the flower is shown in Table 4, in which nomenclature of pistil tissues follows that of Bell and Hicks (1976). All preparations without aniline blue (Fig. 1) and 0 h preparations with aniline blue (Fig. 2) fluoresced a pale green colour when observed with ultra violet light. Bright yellow areas of fluorescence occurred in the stigmatic papillae (Figs. 3 and 4), in the transmitting tissue (Figs. 5 and 6), in the vascular tissue (Figs. 5 and 7) and in the cortex and epidermis (Figs. 8 and 9) from 18 h onwards. In all cases the fluorescence was associated with the cell walls (Figs.

TABLE 4

Percentage of pistils in which aniline blue-positive material was present in the component tissues

Tissue	Time after first opening							
	Unpollinated				Pollinated			
	0 h	18 h	24 h	42 h	0 h	18 h	24 h	42 h
Stigmatic papillae	0	40	86	100	0	50	92	100
Transmitting tissue	0	0	56	100	0	62	86	100
Vascular tissue	0	46	96	100	0	50	88	100
Cortex and epidermis	0	0	16	100	0	0	20	100

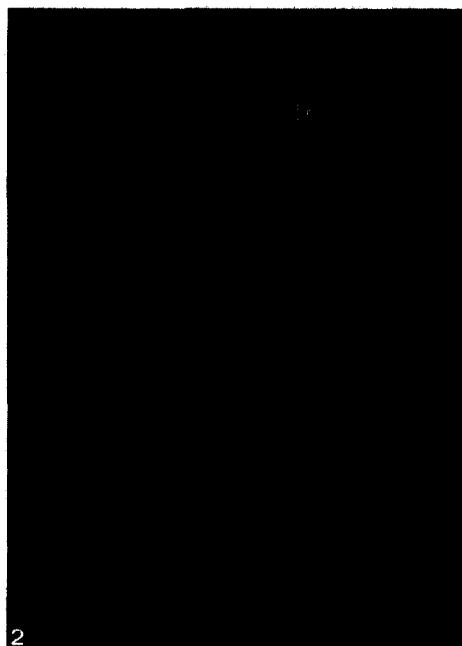
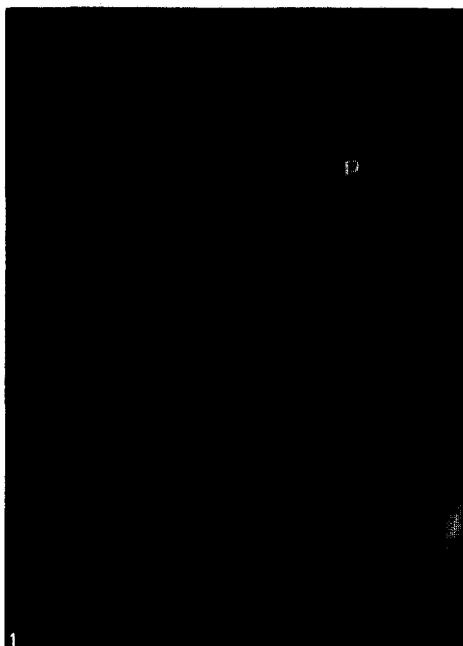


Fig.1. 42 h unpollinated stigma, 10  $\mu$ m section without aniline blue.  $\times$  600. p = papilla cell.

Fig.2. 0 h unpollinated stigma, squash preparation with aniline blue.  $\times$  400. p = papilla cell.

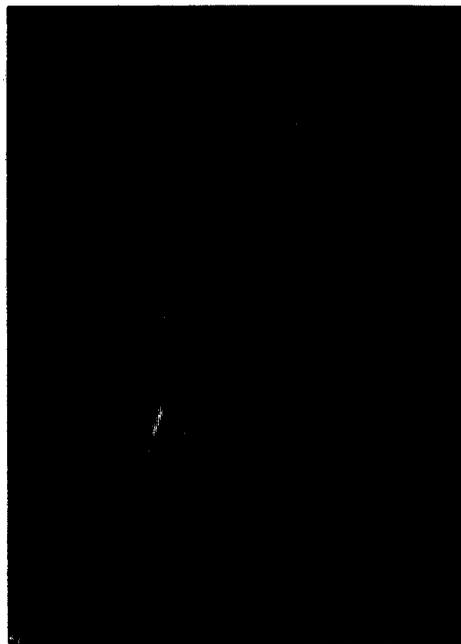
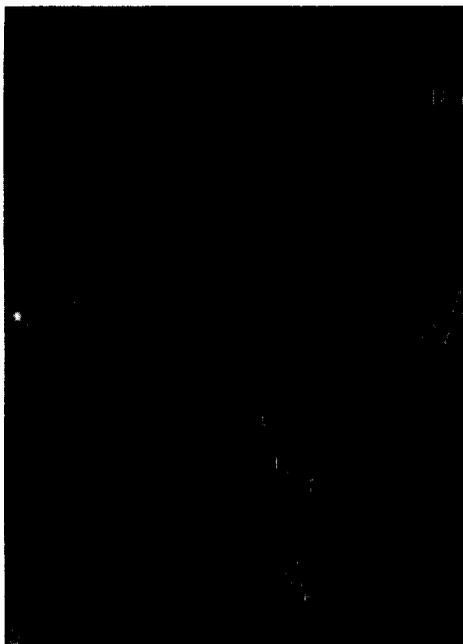


Fig. 3. 18 h unpollinated stigma, squash preparation, with aniline blue.  $\times$  400. p = papilla cell.

Fig. 4. 42 h unpollinated stigma, 10  $\mu$ m section with aniline blue.  $\times$  800. p = papilla cell; c = callose.

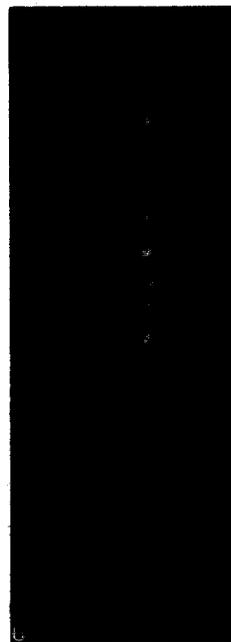
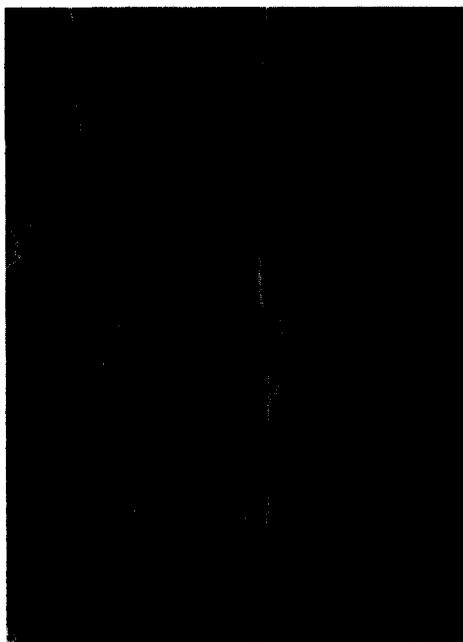


Fig. 5. 24 h pollinated style, squash preparation with aniline blue.  $\times$  75. pt = pollen tube; tt = transmitting tissue; vb = vascular bundle; h = hair.

Fig. 6. 42 h unpollinated style, squash preparation with aniline blue.  $\times$  300. tt = transmitting tissue; c = callose.



Fig. 7. 42 h unpollinated style, 2.25  $\mu$ m section with aniline blue.  $\times$  500. x = xylem; c = callose.



Fig. 8. 42 h pollinated style, squash preparation with aniline blue.  $\times$  75. pt = pollen tube; vb = vascular bundle; co = cortex; c = callose; h = hair.



Fig. 9. 42 h unpollinated style, 10  $\mu$ m section, with aniline blue.  $\times$  500. e = epidermis; c = callose; co = cortex.

TABLE 5

Percentage of unpollinated pistils in which resorcin blue-positive material was present in the component tissues

Tissue	Time after first opening			
	0 h	18 h	24 h	42 h
Stigmatic papillae	0	30	10	70
Transmitting tissue	0	10	20	70
Vascular tissue	0	30	50	80
Cortex and epidermis	0	0	20	100

4, 7 and 9). Callose occurred earlier in pollinated than in unpollinated pistils, particularly in the transmitting tissue. There was no difference in the localisation of fluorescence between preparations collected at the same time which were fixed in Carnoy's fluid or glutaraldehyde.

Staining with resorcin blue gave results similar to aniline blue (Table 5) except that the callose stained blue.

#### DISCUSSION

Although pollen was transferred by direct contact from the anther to the stigma of the male floral stage as observed by Schroeder (1954), penetration of the ovary by a pollen tube did not occur and there was no flower retention. Fewer grains germinated on the stigma at 18 h than at 0 h, and of those that did germinate a smaller proportion grew into the style. Thus the failure to set fruit following pollination of the male floral stage is due, at least in part, to reduced pollen germination and tube growth.

Both aniline blue and resorcin blue have been described as stains for callose (Currier, 1957; Eschrich, 1956), which is a  $\beta$ -1, 3-glucan (Kessler, 1958). However, the physicochemical basis for the staining has not been established (Clarke and Stone, 1963) and Reynolds and Dashek (1976) reported consistent staining of pollen tube walls with lacmoid blue (an analogue of resorcin blue) but not with aniline blue. The two stains used in the present study both gave the same staining pattern and the aniline blue fluorochrome gave more consistent results.

Callose is generally considered to form a barrier as in wounded tissue where it plugs the damaged area (Currier, 1957). Jensen and Fisher (1969) reported its deposition in the transmitting tissue of *Gossypium hirsutum* after passage

of the pollen tubes, as described here in avocado. They suggested that the passage of the tubes changed the permeability of the cells and that the callose was formed as a wound response to prevent cell leakage. Callose occurs around developing pollen (Heslop-Harrison, 1964) and embryosacs (Rodkiewicz, 1973) where it is considered to provide a molecular filter, allowing nutrients to pass but not larger molecules which may interfere with the autonomy of the developing gametophytes. The deposition of callose has also been described in the stigma of flowers with sporophytic self-incompatibility mechanisms such as *Raphanus sativus* (Dickinson and Lewis, 1973). In this case the callose was deposited on the wall of the papilla cell as a result of incompatible pollination and blocked penetration of the stigma by the pollen tube.

In the avocado, reduced pollen tube growth in the pistil of the male floral stage coincided with the appearance of aniline blue-positive and resorcin blue-positive material associated with the cell walls of the component tissues. It is suggested that the presence of callose in the pistil of the avocado may reduce the availability to the pollen of substances necessary for germination and tube growth and thus contribute to the female sterility of the male floral stage.

#### ACKNOWLEDGEMENTS

I wish to thank Mr. D. McE. Alexander for providing the grafted 'Fuerte' plants and Ms. L. Kramm for technical assistance.

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