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EVIDENCE AGAINST THE FORMATION OF FAST DIFFUSING SUBSTANCES PRECEDING FERTILIZATION IN PETUNIA.

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Differentiated protein and RNA metabolism in the ovaries of *Petunia* after cross- and self-pollination has been described previously (LINSKENS 1973, DEURENBERG 1976a, b, 1977). It was observed that the differentiation was altered after pollination long before the tips of the pollentubes reached the ovary. From this observation was concluded that recognition signals had to be emitted from the stigma or the style in order to evoke the differential metabolic processes which result in a differentiated protein metabolism with regard to the total amounts of proteins synthesised as well as the protein pattern.

Two possible explanations were supposed for these phenomena: and electrical signal (LINSKENS & SPANJERS 1973) and fast diffusing substances. Fast diffusing substances are known in the plant kingdom in relation to rhemonastic movements as well as phototropic reactions (UMRATH 1959, LEA 1976).

The style of *Petunia* has two small vascular bundles, differentiated in xylem and phloem which may serve as possible pathways for the transport of the hypothetic signal substances (KONAR & LINSKENS 1966, CANNY 1975; FENSOM 1975). Another possible way of transport are the conductive tissues.

This paper deals with experiments which show evidence against the existence of long distance, fast diffusing signal substances. Pollengrains, labelled with ^{3}H-leucine were brought on the stigma of the *Petunia* flowers. The distribution of the label over the style and the ovary was measured at different time intervals. The *figs. 1* and *2* show that some radio-activity was brought into the stigma, together with the pollen. It can be supposed that the labelled leucine is incorporated into the pollen grains as well as absorbed to the pollen grain-wall (LINSKENS 1959).

In *fig. 1* the distribution of the label over the style, stigma and ovary is shown at 30 minutes, 2 and 4 hours after pollination. In each case the counts per minute had been determined from 2 equivalent pieces of the style or of two stigmas or ovaries.

The distribution of the label over the style is in the same order as the background activity. From this we conclude that no labelled substances were diffused out of the pollen into the style.

A control experiment has been carried out in which a droplet of ^{3}H-leucine solution was brought on the surface of the stigma. Again at several time intervals after administration of the label the style was cut into 2 mm pieces and counted. No diffusion of label over the style was observed. The number of

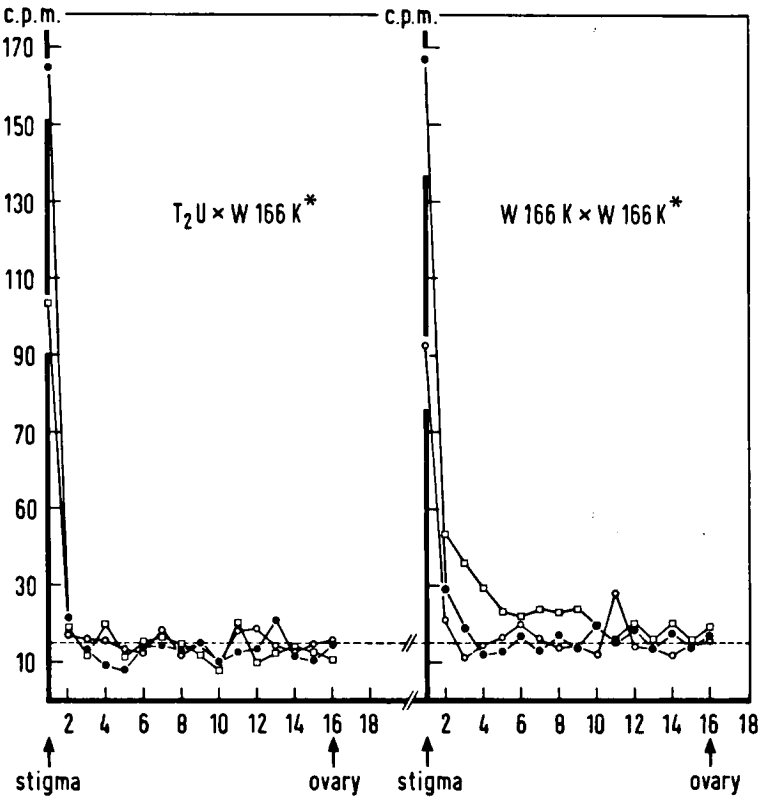

Fig. 1. *Petunia* buds, length of ± 30 mm, from which the corolla and the anthers had been removed, were placed in a vial with a solution containing 1 µl of ^{3}H-leucine (spec. act. 250 mCi/mmol) in 1 ml of water. After incubation for 48 hours at 18°C the pollen was collected, dried and stored in a refrigerator at –20°C.

From buds of 24 hours before anthesis, clone W166K (incompatibility alleles S_1S_2, fig. 1) and clone T_2U (S_3S_3, fig. 2), the petales and the anthers were removed and the remaining ovary, style and stigma with the receptacle was pollinated with the labeled pollen and placed in a vial with a little water. After different time intervals the stigma and the ovary were removed and the style was cut into pieces of 2 mm. All pieces were put into scintillation vials containing 1 ml of Soluene-100 and incubated during 2 hours at 56°C for digestion of the tissues. Ten ml of toluene, PPO and POPOP solution was added and the radio-activity was measured in a Philips Liquid Scintillation Analyser.

□ 30 minutes after pollination.
○ 2 hours after pollination.
● 4 hours after pollination.

counts in each piece of the style did not significantly exceed the background activity.

These results indicate that no substances labelled with ^{3}H-leucine nor ^{3}H-leucine itself possessed the property of fast diffusion and thus can not function

in forwarding information about the kind of pollination from the style or the stigma to the ovary.

These results are in agreement with those of experiments in which labelled proteins were injected in various ways into the style, and/or in which labelled proteins were administered to the surface of the stigma. In those experiments a diffusion of 1 cm per 24 hours had been found (van der Donk, pers. comm.). Such a diffusion is not fast enough since the changes in the protein metabolism of the ovary already take place within a few hours after pollination (DEURENBERG 1976 a,b, 1977).

Although the above mentioned experiments do not exclude the existence of fast diffusing signal substances, it seems that the alternative possibility of signals of an electrical nature is more probable. There are indications that such phenomena can be shown in the style of *Petunia* (LINSKENS & SPANJERS 1973, SINYUKHIN & BRITIKOV 1967).

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