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EFFECT OF SEASON, AGE AND TEMPERATURE ON THE PROTEIN PATTERN OF POLLEN AND STYLES IN PETUNIA HYBRIDA

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SUMMARY

In *Petunia hybrida* age of the plant, temperatures during the vegetative growth and derivation of the plants from summer- or wintercuttings, determine the immunoelectrophoretic patterns of protein in pollen and styles.

The total amount of protein is higher in pollen developed under a higher temperature regime. The quantity of lipids is higher in styles developed under a lower temperature regime.

1. INTRODUCTION

In several plant species pollen tube growth in the style is retarded after incompatible compared with compatible pollination. In connection with this inhibition reaction, differential changes occur in the protein patterns as well as in the activities of several enzymes during the progamic phase (LINSKENS 1955, LINSKENS & TUPÝ 1966, LINSKENS et al. 1969, 1970, ROGGEN 1967, BREDEMEYER 1971, HIRATSUKA & TEZUKA 1980), and in NA-metabolism (LINSKENS 1975, VAN DER DONK 1974a, b). While the way of pollination has an influence on the pollen tube growth in the style, so also does the temperature during (LEWIS 1942, STRAUB 1958, ASCHER & PELOQUIN 1966a, TOWNSEND 1968, LINSKENS 1973, VAN HERPEN & LINSKENS 1981) and before the progamic phase (KWACK 1965, TOWNSEND 1968, DANE & MELTON 1973, VAN HERPEN & LINSKENS 1981). PANDEY (1972) found that the response to a change in temperature was highly specific to particular isozymes and he suggested that temperature induced self-compatibility may result from inactivation or denaturation of specific isozymes or at least one protein involved in the expression of incompatibility in *Lilium*. ASCHER & PELOQUIN (1966a) and HECHT (1964) also suggested that a stylar factor influencing the inhibition of incompatible pollen is heat-labile, a connection between pollen tube growth during and a disturbance of the protein pattern before the progamic phase seems likely.

The age of the plant and of the flower are important factors in the incompatible pollen tube – style interaction (LINSKENS 1964, 1973, HERRERO & DICKINSON 1980, VAN HERPEN & LINSKENS 1981) and in the compatible interaction as well (ASCHER & PELOQUIN 1966b, LINSKENS 1977, VAN HERPEN & LINSKENS 1981). The effect of these factors on the protein pattern has been investigated by PIERARD et al. (1979) who found that aging of the flower of *Sinapis alba* is

attendant upon a change in composition of proteins in leaf or flower primordia and the upper part of the stem. The possibility of aging of the plant and the flower having an effect on the protein pattern of pollen and styles has not yet been investigated. While the effect of temperature, age and season on the pollen tube – style interaction of *Petunia* is known (VAN HERPEN & LINSKENS 1981), the influence of these factors on the protein composition of pollen and styles is not.

2. MATERIALS AND METHODS

The homozygous clone T_2U (S_3S_3) of *Petunia hybrida* was used as pollen and style provider. Unless otherwise stated this self-incompatible clone was grown, from the moment of cutting, in two plant growth chambers, one at 19.5 ± 0.2°C, the other at 25.5 ± 0.2°C during the light period, and both at 18.0 ± 0.1°C in the dark. The light regime was 16 h light (starting at 7.00 a.m.) with a lighting intensity of 25 klx, and 8 h dark.

The plants were grown from cuttings made in August (summer cuttings) and January (winter cuttings) from mother-plants grown in the greenhouse under day-light conditions, in the winter months supplemented with light from a Philips mercury lamp HLRG 400 W, lighting intensity 25 ± 5 klx.

The anthers were collected from flower buds just before anthesis, and dried at 23°C for 24 h in the dark. The pollen was separated from the anther tissue by sieving, and stored at –70°C. Styles were collected from flower buds at the same time as the anthers, immediately frozen in liquid nitrogen, pulverized and stored at –70°C. Pollen and pulverized styles were homogenized in ice-cold PBS (HUDSON & HAY 1976) using a Braun Potter homogenizer (20–30 strokes at 700 r.p.m.); after centrifugation (15,000 × g) the layer of lipids when present was removed and the supernatant was used for immunoelectrophoresis; the protein concentration in the supernatant was measured, after TCA-precipitation, according to LOWRY et al. (1951). Immunization of rabbits, with plant material from the greenhouse, immunoelectrophoresis and preparation and storage of antibody (antiserum) mixture were done according to HUDSON & HAY (1976). Three rabbits were injected with pollen or style proteins (antigens) in concentrations of 1.0–0.5 and 0.1 μg/μl P.B.S. The serum of the three rabbits was mixed to obtain the pollen or stylar antiserum to which all antigens were tested.

Since the barbitone buffer (HUDSON & HAY 1976) gives most pollen and style antigens a net negative charge, the proteins were applied to the cathode side of the immunoelectrophoresis apparatus. The total amount of pollen and style antigen in the upper well was 100 and 60 μg protein, respectively and in the lower well of each slide 70 and 40 μg. The concentration of pollen and style protein was 5.5 and 3.5 μg/μl, respectively, for all slides.

3. RESULTS

The total amount of protein per milligram pollen is higher in pollen developed under a high than under a low temperature regime with or without 24 h of the

Table 1. Effect of temperature and cutting on the protein content of pollen grains. Average of four analyses on 25 mg pollen each, with standard deviation.

Temperature regime during pollen development	mg protein/mg pollen in winter cuttings	mg protein/mg pollen in summer cuttings
19.5/18°C	0.0770 ± 0.0030	0.0795 ± 0.0012
19.5/18°C + 24 h 25.5/18°C 2 to 4 days before anthesis	0.0799 ± 0.0007	0.0800 ± 0.0013
25.5/18°C	0.0991 ± 0.0011	0.1008 ± 0.0017

Table 2. Effect of temperature and cutting on the protein content of styles. Average of four analyses of 65 styles each, with standard deviation.

Temperature regime during style development	mg protein/style in winter cuttings	mg protein/style in summer cuttings
19.5/18°C	0.0560 ± 0.0015	0.0529 ± 0.0031
19.5/18°C + 24 h 25.5/18°C within 3 weeks before maturation of the style	0.0543 ± 0.0017	0.0550 ± 0.0022
25.5/18°C	0.0519 ± 0.0039	0.0521 ± 0.0029

higher temperature (*table 1*). The amount of style protein per style is not significantly different with the applied temperature regimes (*table 2*).

The lipid layer, formed after centrifugation of the homogenized styles, is present when the styles developed under a low temperature regime, and almost absent when the styles developed under one of the other regimes: the high temperature regime or the low one supplemented with 24 h of the high temperature regime. The immunoelectrophoretic pattern of pollen proteins was different depending on whether the plants which delivered the flowers were 14 or 25 weeks old (*fig. 1a, 1c*), (*fig. 1b, 1d*), (*fig. 3a, 3c*), were from winter- or summer-cuttings (*fig. 1a, 3a*), (*fig. 1b, 3b*), (*fig. 1c, 3c*), developed under a low or high temperature regime (*fig. 1a, 1b*), (*fig. 1c, 1d*), (*fig. 3a, 3b*) or a combination of both (*fig. 3c, 3d*). To change the pattern, the pretreatment with 24 h of the higher temperature (*fig. 3d*) has to be given to the plant two to four days before anthesis of the flower to be used; if given earlier (one week before anthesis) the pattern is equal to that of pollen developed under the low temperature regime (*fig. 3c*). The total amount of protein per milligram pollen is higher in pollen developed under a high than under a low temperature regime meaning that some proteins are more concentrated than others leading to a different protein pattern (*fig. 1a, 1b*), (*fig. 1c, 1d*), (*fig. 3a, 3b*). When the concentration of all proteins would have increased to the same extent under the high temperature regime no differences could have been found in the immunoelectrophoretic protein patterns because the amount and concentration of applied antigen is constant (100 or 60 µg, 5.5 µg/µl).

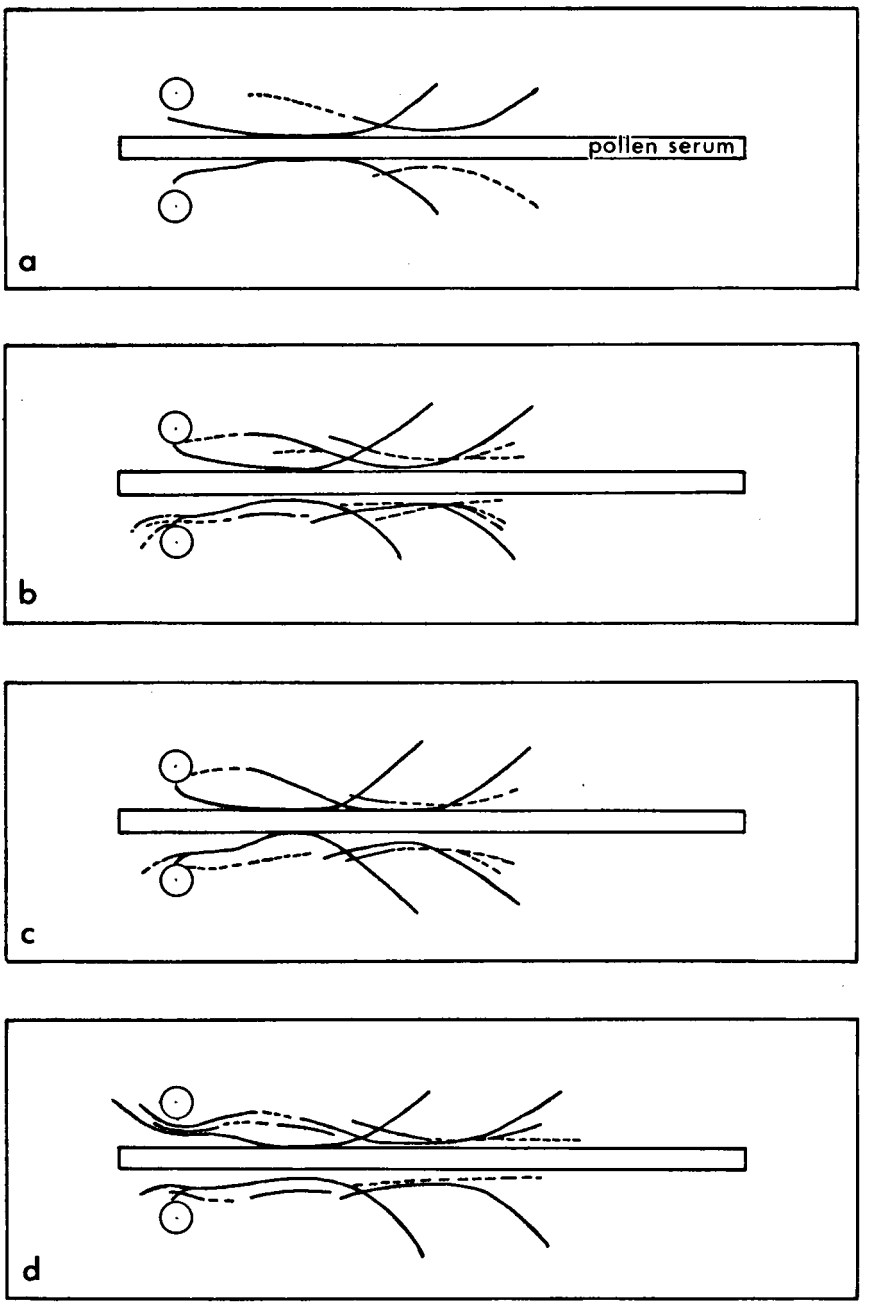

Fig. 1. Immunoelectrophoretic patterns of various pollen antigens, from plants developed from winter cuttings, against pollen serum.
a. Pollen from 14 week old plants grown up at 19.5/18°C
b. Pollen from 14 week old plants grown up at 25.5/18°C
c. Pollen from 25 week old plants grown up at 19.5/18°C
d. Pollen from 25 week old plants grown up at 25.5/18°C

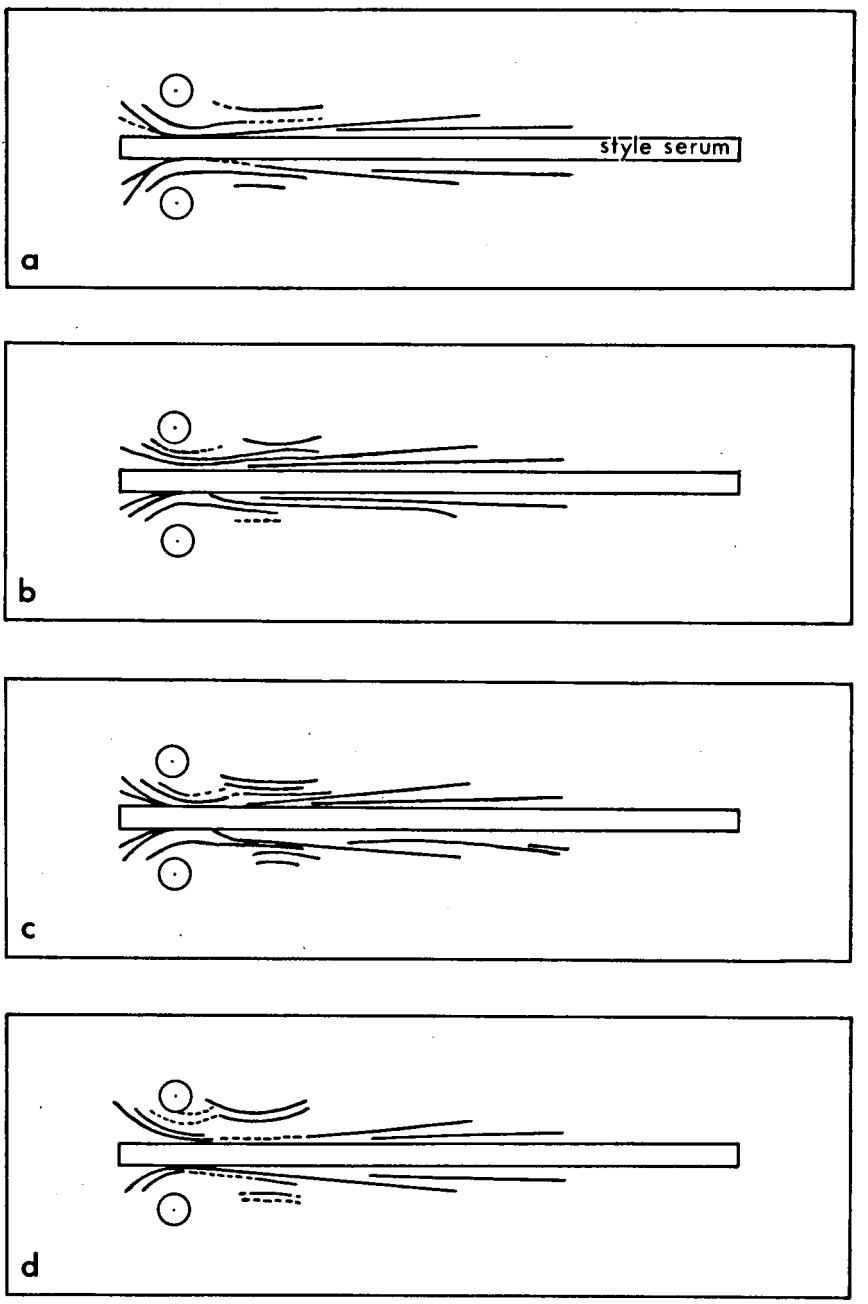

Fig. 2. Immunoelectrophoretic patterns of various style antigens, from plants developed from winter cuttings, against style serum.

a. Styles from 14 week old plants grown up at 19.5/18°C
b. Styles from 14 week old plants grown up at 25.5/18°C
c. Styles from 25 week old plants grown up at 19.5/18°C
d. Styles from 25 week old plants grown up at 25.5/18°C

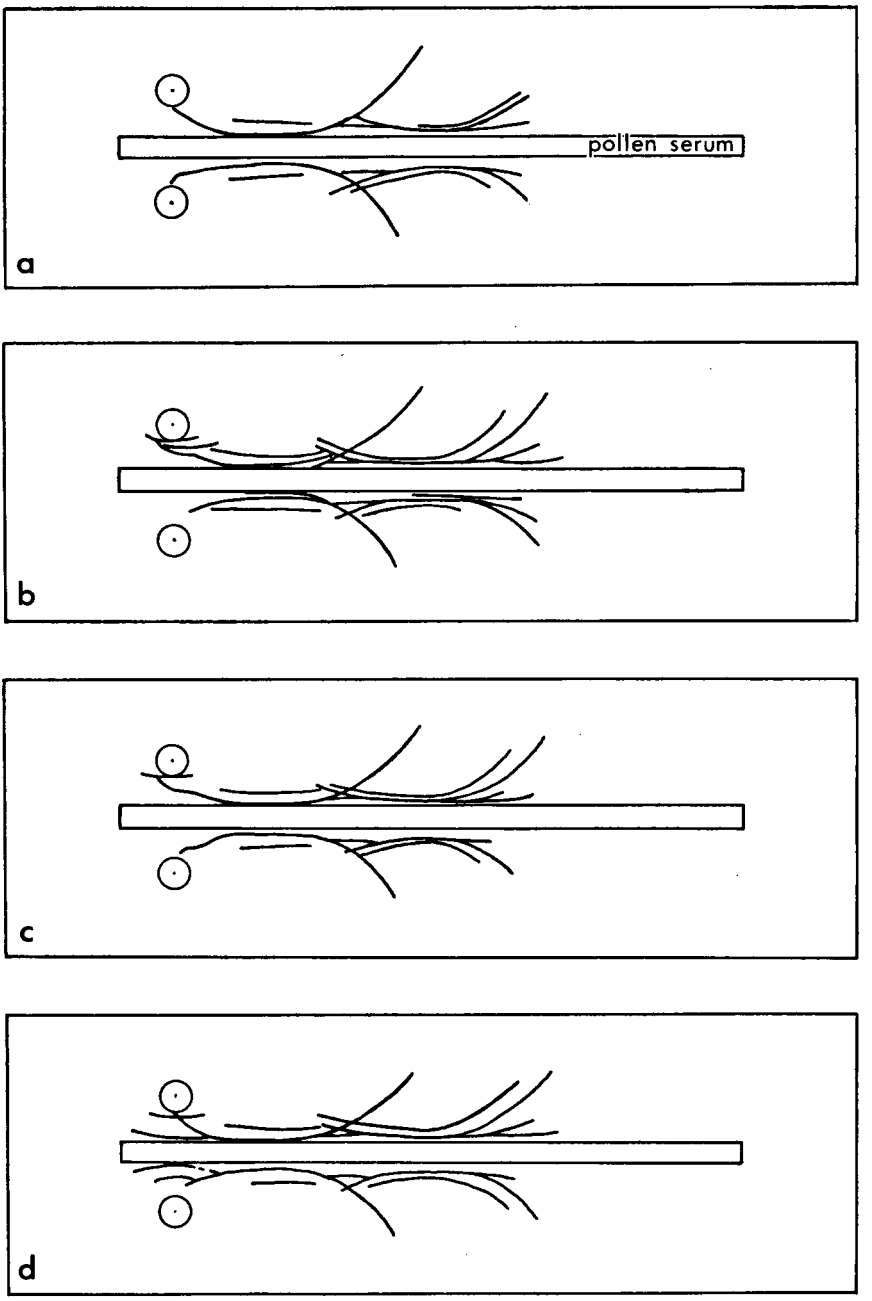

Fig. 3. Immunoelectrophoretic patterns of various pollen antigens, from plants developed from summer cuttings, against pollen serum.
a. Pollen from 14 week old plants grown up at 19.5/18 °C
b. Pollen from 14 week old plants grown up at 25.5/18 °C
c. Pollen from 25 week old plants grown up at 19.5/18 °C
d. Pollen from 25 week old plants grown up at 19.5/18 °C + 24 h 25.5/18 °C on the 2–4th day before anthesis

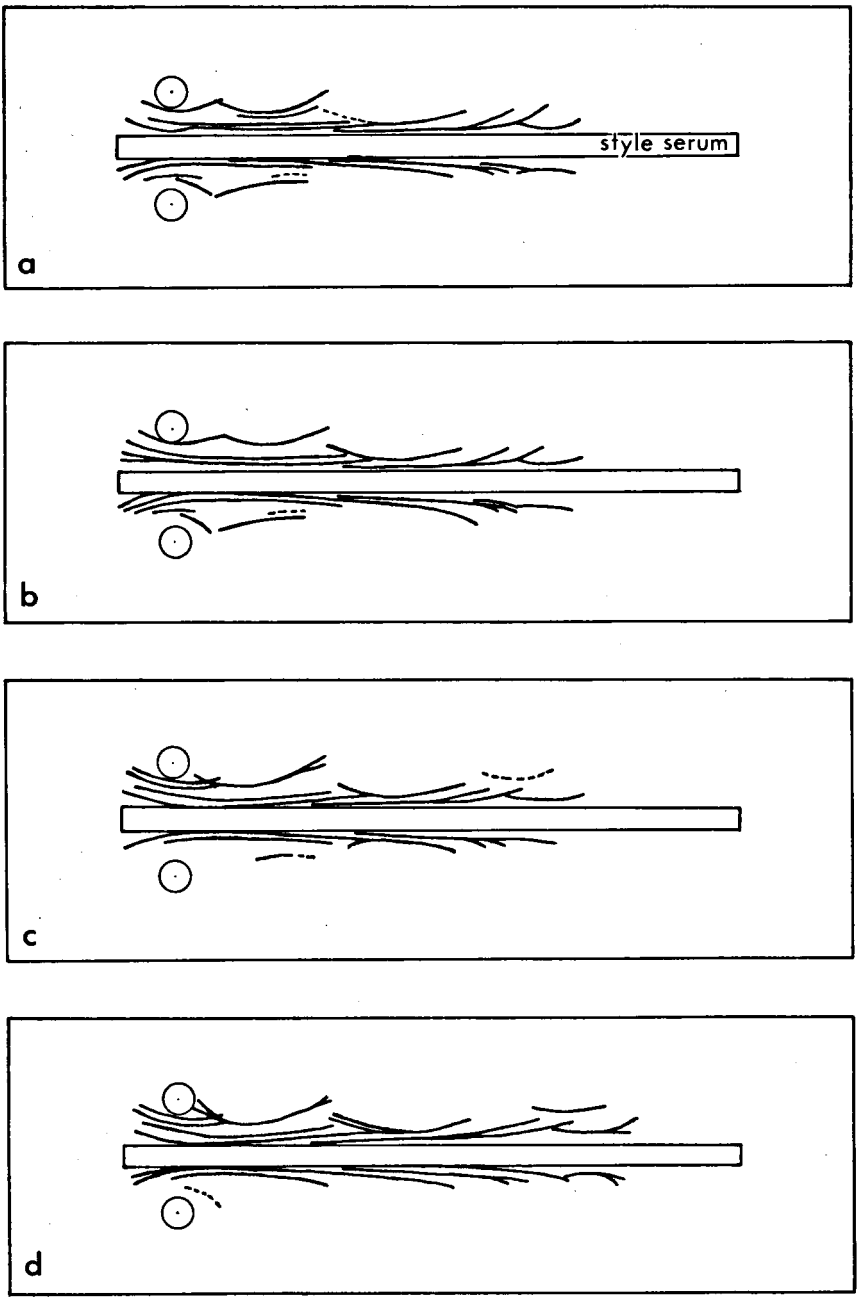

Fig. 4. Immunoelectrophoretic patterns of various style antigens, from plants developed from summer cuttings, against style serum.
a. Styles from 14 week old plants grown up at 19.5/18 °C
b. Styles from 14 week old plants grown up at 25.5/18 °C
c. Styles from 25 week old plants grown up at 19.5/18 °C
d. Styles from 25 week old plants grown up at 19.5/18 °C + 24 h 25.5/18 °C in the week before maturation of the style

The immunoelectrophoretic pattern of precipitation lines from style proteins with stylar serum also was different depending on whether the plants were 14 or 25 weeks old (*fig. 2a, 2c*), (*fig. 2b, 2d*), (*fig. 4a, 4c*), were grown from winter- or summer-cuttings (*fig. 2a, 4a*), (*fig.* 2b, 4b), (*fig. 2c, 4c*), were developed under a low or high temperature regime (*fig. 2a, 2b*), (*fig. 2c, 2d*), (*fig. 4a, 4b*) or a combination of both regimes (*fig. 4c, 4d*). Styles pretreated with 24 h of the higher temperature two to four days before maturation of the style have the same immunoelectrophoretic pattern (*fig. 4c*) as styles pretreated one week before maturation. When the pretreatment was more than three weeks before style maturation, the pattern was similar to that of styles developed under the low temperature regime (*fig. 4c*).

The pattern obtained with a combination of variables is consistent internally. Also, it can be used to predict greenhouse conditions: for the pattern in the laboratory, given a set of variables, is the same as a pattern from pollen and styles from the greenhouse tested against pollen and stylar antiserum if the season of cutting, temperature of growing and plant age in the greenhouse are the same as of the plants in the growth chambers.

4. DISCUSSION

In *Petunia hybrida* incompatible pollen tubes growing in the style attain a greater length in 24 h when the pollen developed, previous to pollination, under a high temperature regime than under a low one (VAN HERPEN & LINSKENS 1981). The different temperature regimes during pollen development can be correlated with the differences in the protein composition of the pollen, as shown by immunoelectrophoresis (*figs. 1,3*), as well as in the total protein per milligram pollen (*table 1*). The incompatible pollen tube growth stops when its resources are consumed (VAN DER DONK 1975), so the observation that incompatible pollen, developed under a high temperature regime, grows farther seems to be confirmed by the fact that its protein content is higher than in pollen developed under a low temperature regime (*table 1*). The length after 24 hours' growth of compatible pollen tubes is enhanced by a pretreatment of the styles, developed under a low temperature regime, with the higher temperature for 24 h. The enhancement is largest when the treatment precedes pollination immediately and is absent when the time between treatment and pollination exceeds four weeks. The pretreatment with the high temperature regime also changes the immunoelectrophoretic pattern of the style proteins. This difference is absent when the time between pretreatment and maturation of the style exceeds three weeks. When two immunoelectrophoretic patterns are the same, it means that only the proteins which form precipitation lines are at the same level of concentration in both slides. A recorded absence of a particular component in the pattern indicates that the causal protein is no longer present at the level required for precipitation band formation, but that protein may be present still at a higher or lower level than before. Age of the plant and cutting have an impact on the compatible and incompatible pollen tube growth during the progamic phase (VAN HERPEN &

LINSKENS 1981) and they both have a comparable effect on the protein pattern of pollen and styles (*figs. 1, 2, 3, 4*).

The fact that temperature pretreatments, cutting and age of the plant have an effect on the pollen tube length as well as on the protein pattern of pollen and styles (*figs. 1, 2, 3, 4*) before the progamic phase, and that the self-incompatibility mechanism in *Petunia* is built up during the course of flower development and begins to express itself just before bud opening, with specific synthesis of RNA and protein (LINSKENS 1966, KOVALEVA et al. 1978) allows the conclusion that a relation between alteration of protein composition and different pollen tube lengths, due to environmental and physiological conditions, is likely. Whether or not it is a function of the S-gene, the gene activity and subsequent polypeptide synthesis before the progamic phase is independent of the kind of pollination. The differential gene activity VAN DER DONK (1974a, b) found is in my opinion a different gene activity influenced by the kind of pollination and is perhaps the result of the gene(s) working before pollination. VAN DER DONK (1975) claims the synthesis of style specific polypeptides during the progamic phase. The change in protein composition before the progamic phase can have an effect during the progamic phase when those proteins are necessary for the activation of the style or for the interaction with target proteins or protein masking mRNA (VAN DER DONK 1975). If the synthesis of specific style polypeptides (VAN DER DONK 1975) is not restricted to the progamic phase, changes in protein composition before the progamic phase are perhaps caused by changes in the synthesis of those specific polypeptides. It is also possible that environmental and physiological conditions influence the pollen tube – style interaction either via a change in structure and/or distribution of lipids in the membranes (FURTH 1980, DELBART et al. 1980) or via a change in the stylar metabolites (ASCHER 1966). The fact that the turn-over of lipids can be achieved after 24 h of a high temperature regime means that enzymes have to be activated which are perhaps responsible for the change in protein pattern.

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