environment and pollen style interaction

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Relatie tussen omgevingsvariabelen voor en tijdens de bestuiving, de daaraan gekoppelde biochemische veranderingen in pollen en stijl en de invloed hiervan op de lengte van de pollenbuizen na de bestuiving.

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INTRODUCTION

In the *Angiosperms* we distinguish three different barriers which prevent either fertilization or the production of a fertile progeny incongruity, incompatibility and sterility

Processes towards fertilization together with zygote development in higher plants are based on interactions between (HESLOP- HARRISON & LINSKENS 1984) and within cells (LINSKENS 1980) The barriers can be classified according to the site of reaction Incompatibility is a fertilization barrier during the progamic phase, i e the period between the landing of the pollen on the stigma and the double fertilization It is based only on disturbances of the intercellular interactions between pollen and style

Sterility is related to all the disturbances of interactions within the cells of the embryo sac after the progamic phase has ended, while incongruity acts on inter- as well as on intra-cellular interactions before and after fertilization, respectively

For a successful progress of the progamic phase it is a necessity that pollen and style have matching genic systems (HOGENBOOM 1975); based on this formulation a distinction can be made between incongruity and incompatibility.

Incongruity or interspecific incompatibility deals with the non-functioning of an intimate partner relationship resulting from a lack of genetic information in one of the partners about some relevant character of the other (HOGENBOOM 1984), the partners do not fit together (HOGENBOOM 1975).

Incompatibility or intraspecific incompatibility frustrates the functioning of the pistil-pollen relationship, though the potential for functioning of the partners is complete (HOGENBOOM 1984). The disturbing principle of incompatibility results from identical genes (in e g *Petunia*, our object of study, S-genes) and their specific products (LEWIS 1965, LINSKENS 1968, HESLOP-HARRISON *et al* 1975, LEWIS 1979, FERRARI *et al* 1981) Specific pollen wall proteins are released during the stay of the pollen on the stigma They probably have a function as recognition substances (HESLOP-HARRISON 1971, KNOX & HESLOP-HARRISON 1970) and interact with complementary molecules of the other partner (LINSKENS 1953, 1955, MAKINEN & LEWIS 1962, NASRALLAH *et al* 1970, VAN DER DONK 1975, HESLOP-HARRISON 1978, HERRERO & DICKINSON 1980, BREDEMEIJER & BLAAS 1981)

There are two classes of proteins in pollen walls (KNOX & HESLOP-HARRISON 1971) exine proteins produced by the diploid tapetal tissue, the sporophyte on one side and intine proteins synthesised by the haploid pollen, the sporophyte on the other side This gives rise to two different genetic systems which disturb the normal pollen-pistil interactions during the progamic phase sporophytic- and gametophytic- incompatibility

In a sporophytic system, mostly found in the tricellular pollen, the pollen does not germinate or the pollen tubes fail to penetrate the stigma surface, or penetrate only a short distance. In most gametophytic systems studied, the bicellular pollen grains (except the tricellular pollen of the *Gramineae*) germinate in a normal way and produce pollen tubes which penetrate the stigma and grow down into the style where growth stops at various depths.

The recognition process in a gametophytic incompatibility system, as in *Petunia hybrida*, is delayed in comparison with a sporophytic system. This is probably because of the fact that the recognition molecules has to come out of the intine of the pollen wall instead of the exine as in a sporophytic situation.

On the other hand the complementary recognition molecules in the style are probably also proteinous in nature (LINSKENS 1953, 1955, MAKINEN & LEWIS 1962, NASRALLAH *et al.* 1970, VAN DER DONK 1975, HERRERO & DICKINSON 1980, BREDEMEIJER & BLAAS 1981).

The pollen-style recognition process itself is specific (DE NETTANCOURT 1977) because it depends on the S-genes and their specific molecules and finally results in acceptance or non-acceptance of the pollen tubes after a compatible or incompatible pollination, respectively (VAN DER DONK 1975).

The mechanisms of the recognition and follow-up-reaction is not known. Their expression can be visualized in the length of the pollen tubes after pollination. But this length can also be influenced by environmental conditions during the progamic phase (LEWIS 1942, STRAUB 1958, KWACK 1965, ASCHER & PELOQUIN 1966a,b, TOWNSEND 1968, DANE & MELTON 1973, LINSKENS 1973, DEURENBERG 1977, GILISSEN 1978, HERRERO & DICKINSON 1980). The effect of environmental factors during pollen and style development on the processes in the progamic phase is hardly investigated. Our goal was the search for a component that is part of, or related with the chain of reactions from recognition on towards acceptance or non-acceptance of the pollen tubes. The best component would be the one which presence or action could be influenced by certain environmental conditions.

So first we determined the effect of different environmental conditions during the progamic phase as well as during pollen and style development, on the ultimate length of the pollen tubes after 24 hours growth on the style (VAN HERPEN & LINSKENS 1981). It proved that the expression of the compatible and incompatible pollination could be influenced by the temperature during the development of the style and pollen, respectively.

Secondly we tried to make a correlation between the temperature during pollen and style development, the compounds present in pollen and style before pollination and the expression of the incompatibility and compatibility reaction during the progamic phase. We looked for quantitative and/or qualitative changes in proteins, lipids and free

carbohydrates (low molecular weight carbohydrates) in pollen and styles as a function of the applied temperature before pollination Correlation of those biochemical alterations with the length of the pollen tubes after pollination pinpointed to style molecules with a proteinous character (VAN HERPEN 1981, 1983) as far as the compatible pollination is concerned

Thirdly we tried to test the effect of those compounds, synthesized in the style before pollination when the temperature during style development was high enough, on growing pollen tubes in a *semi-vitro* system In this experiment style polypeptides proved to be important in controlling pollen tube length (VAN HERPEN 1984) These polypeptides can be part of the recognition- or follow-up-reaction If the recognition process depends only on the kind of pollination (VAN DER DONK 1975) these polypeptides have to be part of the follow-up-reaction Recognition is then clearly separated from some step in the follow-up-reaction

Fourthly this division can be useful in studying recognition as we did with a modified but essentially the same colorimetric method as described by PRAT (1962)

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REFERENCES

ASCHER, P D & S J PELOQUIN (1966a) *Am J Bot* **53** 99-102
— & — (1966b) *Can J Genet Cytol* **8** 661-664
BREDEMEIJER, G M M & J BLAAS (1981) *Theor Appl Genet* **59** 185-190
DANE, F & B MELTON (1973) *Crop Sci* **13** 587-591
DEURENBERG, J J M (1977) Thesis, University Nijmegen
DONK, J A W M VAN DER (1975) Thesis, University Nijmegen
FERRARI, T E , S S LEE & D H WALLACE (1981) *Phytopathology* **71** 752-755
GILISSEN, L J W J (1978) Thesis University Nijmegen
HERPEN, M M A VAN & H F LINSKENS (1981) *Acta Bot Neerl* **30** 209-218
—(1981) *Acta Bot Neerl* **30** 277-287
—(1983) *Proc 7th Int Symp on Fertilization and Embryogenesis in Ovulated Plants*, pp 227-230 14-17th June 1982 Račkova Dolina, Czechoslovakia
—(1984) *Acta Bot Neerl* **33** 195-203
HERRERO, M & H G DICKINSON (1980) *Planta* **148** 138-145
HESLOP-HARRISON, J (1971) *Pollen development and physiology*, pp 75-98 (Ed J HESLOP-HARRISON) Butterworths, London
—(1978) *Studies in Biology* **Vol.100** London, U K
—(1983) *Proc R Soc Lond* **B218** 371-395
—, Y HESLOP-HARRISON & J BARBER (1975) *Proc R Soc Lond* **B188** 287-297
— & H F LINSKENS (1984) *Encyclopedia of Plant Physiology* **Vol. 17.** Pp 2-17
HOGENBOOM, N G (1975) *Proc R Soc Lond* **B188** 361-375
—(1984) *Encyclopedia of Plant Physiology* **Vol. 17.** Pp 640-654
KNOX, R B & J HESLOP-HARRISON (1970) *J Cell Sci* **6** 1-27
— & —(1971) *J Cell Sci* **9** 239-251

KWACK B H (1965) *Physiol Plant* **18** 297-305
LEWIS, D (1942) *Proc R Soc Lond* **B131** 13-26
—(1965) *Genetics Today* **3** 657-663
—(1979) *Studies in Biology* **Vol. 110.** London, U K
LINSKENS, H F (1953) *Naturwissenschaften* **40** 28-29
—(1955) *Z Bot* **42** 1-44
—(1968) *Quad Acc Naz Dei Lincei (Roma)* **104** 47-56
—(1973) *Fiziol Rast* **20** 192-203
—(1980) *The cell surface mediator of developmental processes* Eds SUBIELNY, S & N K WESSELS Academic Press, London, New York, pp 113-126
MAKINEN, Y L A & D LEWIS (1962) *Genet Res* **3** 352-363
NASRALLAH, M E , J T BARBER & D H WALLACE (1970) *Heredity* **25** 23-27
NETTANCOURT, D DE (1977) *Incompatibility in Angiosperms* Springer-Verlag, Berlin-Heidelberg-New-York
PRAT, H (1962) *Moderne Methoden der Pflanzenanalyse* **5** 359-367 (Eds LINSKENS, H F & M V TRACEY) Springer-Verlag, Berlin-Gottingen-Heidelberg
STRAUB, J (1958) *Z Bot* **46** 98-111
TOWNSEND, C E (1968) *Crop Sci* **8** 269-272

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EFFECT OF SEASON, PLANT AGE AND TEMPERATURE DURING PLANT GROWTH ON COMPATIBLE AND INCOMPATIBLE POLLEN TUBE GROWTH IN PETUNIA HYBRIDA

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SUMMARY

In *Petunia hybrida* the length of pollen tubes was measured after growth in the style for 24 h The length attained depends upon the temperature regime under which the pollen as well as the styles developed prior to pollination The temperature regimes used were 25 5/18 C and 19 5 18 c respectively, both in a 16/8 h day night regime

A high temperature regime during the progamic phase causes a greater length of the tubes than the low temperature regime, this difference is larger after cross- than after self-pollination

The length after 24 hours growth of incompatible pollen tubes is larger when the pollen developed, prior to pollination, under the high temperature regime than under the low one, but their length is not affected by the temperature at which the styles developed With compatible pollen tubes, on the other hand that length is larger when the styles developed under the high temperature regime than under the low one and it is also enhanced by a pretreatment of styles developed under a low temperatue regime with the higher temperature for 24 h The enhancement is largest when the treatment precedes pollination at the low temperature regime immediately and is absent when the time between treatment and pollination exceeds four weeks

The length of incompatible pollen tubes after 24 h is in general lower in plants cultivated from summer- than from winter-cuttings the length of compatible pollen tubes in plants cultivated from winter-cuttings decreases with the age of the plant, if style development and tube growth take place under the low temperature regime

Compatible pollen germination is affected by a low temperature regime during style development and progamic phase

The incompatible pollen germination rate is lower in pollen from summer- than from winter-cuttings, when pollen development and pollination occur at the low temperature regime

1 INTRODUCTION

In several plant species the growth of the pollen tubes in the style is much slower after an incompatible pollination than after a compatible one This growth inhibition weakens towards the end of the flowering period of the plant producing the styles (LINSKENS 1973) In young flower buds the growth inhibition capacity of the style is weak, but during maturation it increases (LINSKENS 1964, HERRERO & DICKINSON 1980) In old and wilting flowers this growth inhibition reaction decreases again (ASCHER & PELOQUIN 1966a) Age is an important physiological factor not only in relation to the incompatible pollen tube – style interaction, but also in relation to the compatible one ASCHER & PELOQUIN (1966a) found in *Lilium* that compatible pollen tube growth velocity varies with the physiological age of the styles LINSKENS (1977) investigated compatible

pollen tube lengths during the flowering period of *Petunia* plants, but it is not clear whether the differences observed were caused by the advancing age of the plants or by the advancing growth season.

The temperature is important during the progamic phase (LEWIS 1942, ASCHER & PELOQUIN 1966b, TOWNSEND 1968, STRAUB 1958, LINSKENS 1973), but also before: in *Oenothera* a partial decrease of the incompatibility reaction was obtained by either a low temperature during, or a high temperature prior to the progamic phase (KWACK 1965). Low temperatures before pollination had no effect on the incompatible pollen tube growth in *Oenothera* (LEWIS 1942), but changed the compatibility reaction in *Trifolium* to self-incompatibility (TOWNSEND 1968). However, in *Medicago sativa* pollen germination was hardly affected by incubation temperatures, but more by the temperature at which the plants were grown (DANE & MELTON 1973).

Still less is known about the impact of temperature pretreatments on the pollen tube – style interaction. We decided therefore to investigate the effect of temperature treatment during the pre-progamic phase and of aging and season in relation to the pollen tube – style interaction after compatible or incompatible pollinations.

2. MATERIALS AND METHODS

The heterozygous clone W166K (S_1S_2) of *Petunia hybrida* was used only as pollen provider and the homozygous clone T_2U (S_3S_3) for the pollen and styles.

Unless otherwise stated both self-incompatible clones were 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.), lighting intensity 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; it was used immediately for pollination by applying an excess on the stigma. Styles of emasculated flowers were pollinated in both growth chambers at 9.00 a.m. and after 24 h (progamic phase), both for cross- and self-pollinations the length of the pollen tubes was measured with an U.V. fluorescence microscope according to the method of LINSKENS & ESSER (1957). Eight styles were selected at random and the average length of 90% of the pollen tubes was determined.

Experiments could be carried out from the 10th till the 25th and the 15th till the 25th week for summer and winter cuttings respectively.

3 RESULTS

The vegetative vigour of the plants in the growth chambers was better than that of plants in the greenhouse of the same age The plants grown under the low temperature regime were more vigorous than those grown under the high temperature regime

3 1 Self-pollination

After self-pollination ($S_3S_3 \times S_3$) we got the following results depending on the applied pretreatment

Temperature during style development The length of incompatible pollen tubes is not correlated with the temperature at which the style developed (*fig 1*), not even when the temperature during pollination is high (*fig 2*)

Temperature during pollen development The tube is longer when the pollen developed at the high temperature regime (*fig 1, 2*)

Temperature during the progamic phase A comparison of *figs 1* and *2* shows that the high temperature has an equal impact on the two curves in figure 1 except that there is no decrease in length after the 21st week

Aging of the plant The pollen tube length and germination percentage appear independent of the age of the plant except for the 19.5, 18 C temperature regime during pollen development and progamic phase (*fig 1*) at which pollen tube length is smaller and the germination percentage lower after the 21st week

Growth-season In summer cuttings the pollen tube length is in general lower than in winter cuttings The percentage of germination is lower in the former than in the latter, but only after the low temperature regime during pollen development and progamic phase

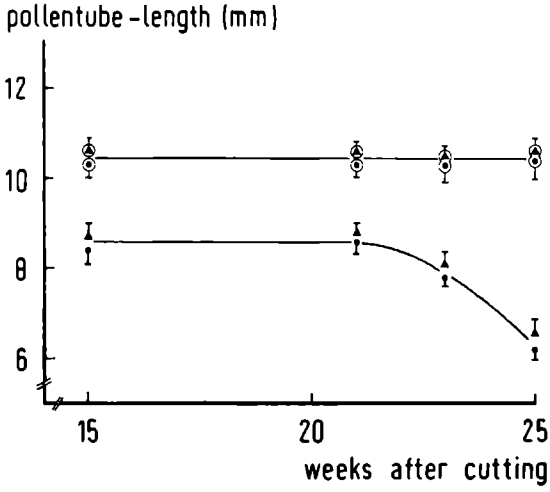

Fig 1 Length of incompatible pollen tubes, from pollen developed at 25 5 18 C (◉ ⊙) and 19 5 18 C (▲ ●) after 24 hours growth at 19 5 18 C in styles developed at 19 5 18 C (◉, ▲) and 25 5 18 C (⊙, ●) Pollen and styles are from plants developed from winter cuttings

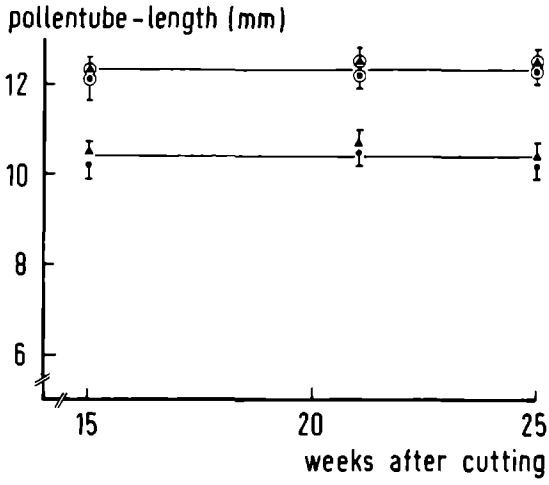

Fig 2 Length of incompatible pollen tubes, from pollen developed at 25 5 18 C (▲, ⊙) and 19 5 18 C (▲, ●), after 24 hours growth at 25 5 18 C in styles developed at 19 5 18 C (▲, ▲) and 25 5 18 C (⊙, ●) Pollen and styles are from plants developed from winter cuttings

Figs. 1 and *2* are similar for the summer cuttings except that experiments could be started five weeks earlier when summer cuttings were used. Tubes, from pollen developed at the high temperature regime, attain a greater length than tubes from pollen developed at the low temperature regime no matter if we used summer- or winter-cuttings.

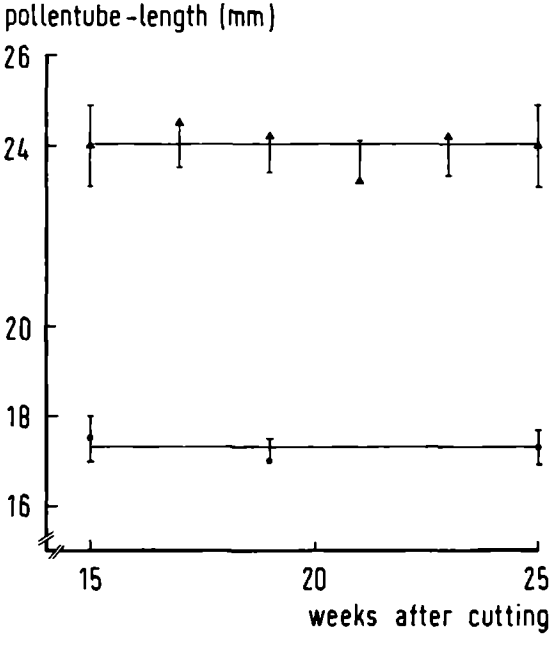

Fig 3. Length of compatible pollen tubes, from pollen developed at 25 5 18 C, after 24 hours growth at 25 5/18–C (▲) and 19 5 18 C (●), in styles developed at 25 5/18 C Pollen and styles are from plants developed from winter- or summer-cuttings

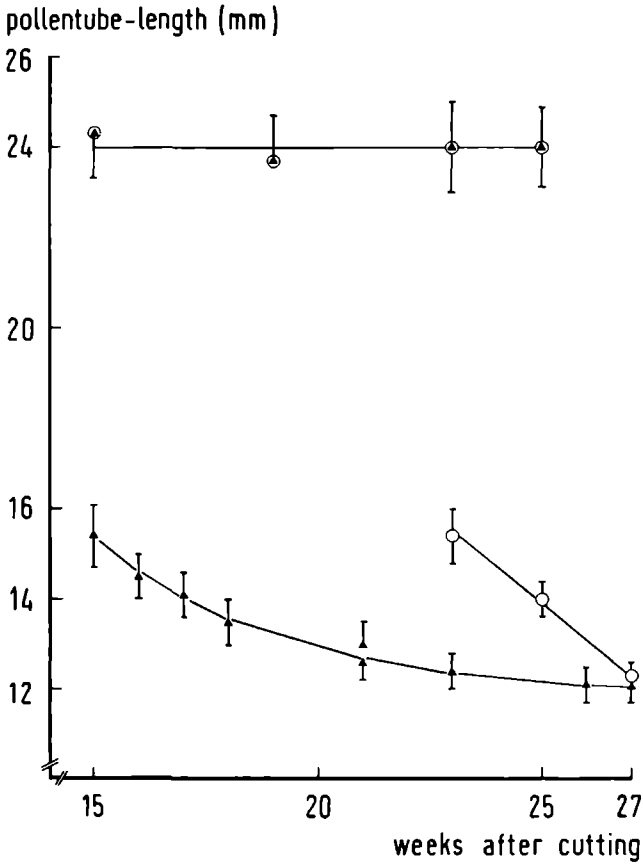

Fig. 4 Length of compatible pollen tubes, from pollen developed at 25 5 18 C, after 24 hours growth at 25 5 18 C (▲) and 19 5 18 C (▲, ○) in styles developed at 19 5 18 C (▲, ▲) and 19 5 18 C + in the 23the week 24 hours 25,5/18 C just before the start of the progamic phase (○). Pollen and styles are from plants developed from winter cuttings

3.2. Cross-pollination

After cross-pollination (S_3S_3 × S1,2) the following observations for the different pretreatments are made.

Temperature during style development: Compatible pollen tubes grown at 19.5/18 °C are longer in styles developed at 25.5/18 C than in styles developed at 19.5/18 °C (*fig. 3, 4* and *5*). In styles developed at 25.5/18 °C and those developed at 19.5/18 C the behaviour of the tubes raized after pollination at 25.5/18 °C, are with regard to the length of the compatible tubes identical (*fig. 3, 4*). When an exposure of only 24 hours of the high temperature regime was given to the styles before the progamic phase, the length of the compatible tubes is larger in those styles compared with non-exposed styles (*fig. 4, 5*) provided that the temperature during style development and pollination is low. The time lag between exposure

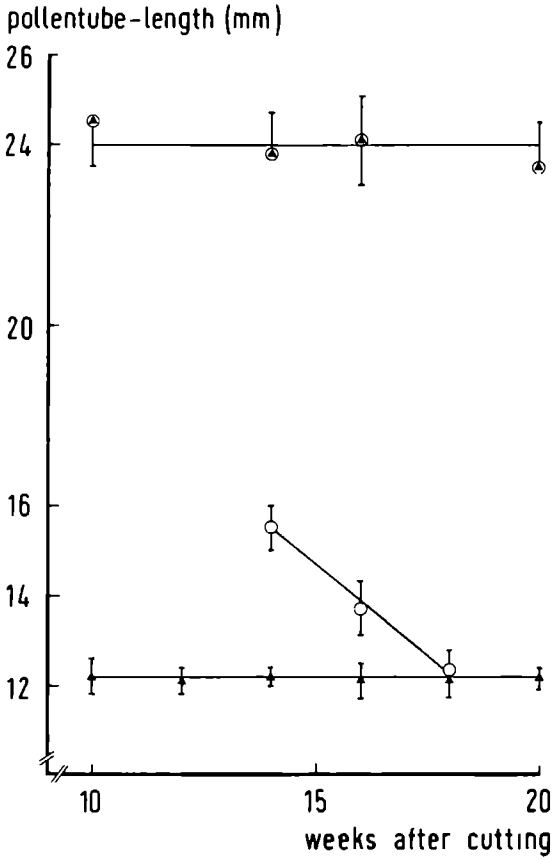

Fig 5 Length of compatible pollen tubes, from pollen developed at 25 5 18 C, after 24 hours growth at 25 5 18 C (▲) and 19 5 18 C (▲, ○), in styles developed at 19 5 18 C (▲, ▲) and 19 5 18 C + in the 13th week 24 hours 25 5 18 C just before the start of the progamic phase (○) Pollen and styles are from plants developed from summer cuttings

and the moment of pollination is important the length of the tubes becomes shorter when time between pretreatment and start of the progamic phase increases (*fig. 4, 5*).

Temperature during pollen development The pollen tube length after cross-pollination is not significantly different from pollen developed at different temperature regimes

Temperature during the progamic phase The compatible tube length is larger and the germination percentage higher in styles developed and pollinated at 25 5/18°C or developed at 25.5/18 C and pollinated at 19 5/18°C than in styles developed and pollinated at 19.5/18 C (*fig 3, 4, 5*)

Aging of plants The effect of aging is only visible in winter cuttings when both temperatures during style development and progamic phase are 19 5/18°C (*fig. 4*)

Season effects: In winter cuttings the pollen tubes grow a shorter distance with increasing age of the plant (*fig. 4*) when both temperatures during pollination and style development are 19.5/18 °C. The length after 25 weeks is equal to the length in summer cuttings after 10 weeks (*fig. 4, 5*).

4. DISCUSSION

Pollen tube growth depends not only on the temperature regime during the progamic phase but also on the temperature regime during the preceding flower and plant development. The effect of temperature during pollen or style development on the lengths of incompatible or compatible pollen tubes respectively can be discussed in relation to the most fitting gene action models of self-incompatibility put forward by ASCHER (1966) and VAN DER DONK (1975). The process of recognition after the landing of pollen on the stigma leads to a response in the pistil (LINSKENS 1975) and the time lag between the start of the progamic phase and rejection or acceptance of the pollen in the pistil can be controlled by the temperature during the progamic phase (LEWIS 1952). We think that the low temperature regime during style development and progamic phase does not only prolong the recognition process in *Petunia*, but also alters it in such a way that acceptance of the compatible pollen does not occur, and that this probably explains why the compatible pollen tubes have approximately the same length as the incompatible ones (see also ASCHER & PELOQUIN 1966b for *Lilium*). According to the model of VAN DER DONK (1975), the style specific polypeptides are probably not synthesized by the stylar S-gene, because either there is no activation of the style or the style does not have preformed mRNA, and thus, as in the case of an incompatible pollination, the pollen genome is not activated (since there are no style specific polypeptides), and subsequently the pollen tube growth stops when its resources, depending on the kind of pollen (T_2U or W166K) and the temperature during pollen development, are consumed. The observation that incompatible pollen, developed at a higher temperature, grows farther due to its resources seems to be confirmed by the fact that its protein content is higher than in pollen developed at a lower temperature regime (VAN HERPEN in prep.). According to the model of ASCHER (1966), the high velocity operon is not repressed, but the high velocity growth system cannot move into action because the necessary stylar metabolites are not available because of the low temperature regime during style development and progamic phase, thus growth of the pollen tube occurs at the slow rate until compatible pollen reserves have been depleted.

The difference in compatible tube lengths in plants cultivated at the low temperature regime either from cuttings made in summer or in winter cannot be explained by a difference in pollen germination percentage (see TER-AVANESIAN 1978), but perhaps by a different stylar gene activity (VAN DER DONK 1975, LINSKENS 1975) towards fast repair of the damage caused by the growing pollen tube.

All styles mentioned above are developed and pollinated at the low tempera-

ture regime, higher temperatures during the progamic phase induce longer incompatible pollen tubes in *Petunia* (LINSKENS 1973), *Trifolium* (CHEN & GIBSON 1973, TOWNSEND 1968), *Lilium* (ASCHER & PELOQUIN 1966b) and *Brassica* (GONAI & HINATA 1971) Our experiments in the growth chambers give similar results for compatible pollen tubes as found by ASCHER & PELOQUIN (1966b), except that compatible tubes have approximately the same length as incompatible tubes at the low temperature regime and twice as long at the high temperature regime during pollination According to the model of VAN DER DONK (1975), the style specific polypeptides are probably synthesized very quickly by the high pollination temperature and either do not or do react whether it is a compatible or incompatible pollination, with the pollen specific polypeptides A differential reaction takes place (VAN DER DONK 1974) leading to a large difference in the respective pollen tube lengths In the model of ASCHER 1966) the high velocity operon is not repressed, as in the case where low temperatures during style development and progamic phase are used, but the high-velocity system can move into action now because the stylar metabolites are synthesized very quickly by the high temperature regime during the progamic phase

When styles are developed under the low or high temperature regime and pollinated at the low temperature regime, the tube lengths are only different for compatible and not for incompatible pollinations This difference in compatible pollen tube lengths occurs also when styles developed at 19.5/18°C are treated with 24 hours of the high temperature regime before the start of the progamic phase as compared with styles which are not, and this difference lasts for about four weeks when pollination is still performed at the low temperature regime VAN DER DONK (1975) claims the synthesis of style specific polypeptides during the progamic phase Our results suggest either temperature dependent S-gene activity and subsequent polypeptide synthesis before the progamic phase or S-gene activity during the progamic phase but affected by temperatures before the progamic phase, the mechanism of this last possibility is, however, not yet clear In the model of ASCHER (1966), enough stylar metabolites are synthesized during the high temperature pretreatment to enable the action of the high-velocity system even when the pollination temperature is low The length of incompatible pollen tubes is independent of the temperature during style development, and dependent on the temperature during pollination but not to such an extent as the compatible tubes are In the model of ASCHER (1966) the latter observation can be explained by the fact that there are different operons at work for the different pollinations, and in the model of VAN DER DONK (1975) by a gene, responsible for the repair of the damage caused by the growing pollen tube, which works only during incompatible pollinations and better at a high temperature

Germination of alfalfa (*Medicago sativa*) pollen is dependent upon the clone from which the pollen is collected and influenced by the temperature at which the plants are developed (DANE & MELTON 1973) HAYASE (1955) found maximum germination of pollen from male flowers of cucumber at 20–25°C, lower and higher temperature treatments given from 2 days before anthesis decreased the pollen viability. The decrease in the percentage of pollen germination and pollen

tube length in *Petunia* especially at the low temperature regime is perhaps caused by a change in the calcium concentration (BRLWBAKER & KWACK 1963) in the style The pollen tube length is also determined by the number of germinated pollen grains (TER-AVANESIAN 1978)

After self- or cross-pollination a change in the saturation degree of the fatty acids is found, and the distribution of the fatty acids among the various glycosphingolipids in self- and cross-pollinated styles is different (DELBART et al 1980) Bacteria, plants and animals can all adapt to changes in environmental temperature by altering the degree of saturation in their fatty acid side chains (FURTH 1980) It is possible that environmental conditions before and during the progamic phase have their influence on the pollen tube – style interaction via a change in structure and/or distribution of lipids in the membranes

Environmental factors are not only decisive *during* the progamic phase but also *before* Thus the use of controlled conditions for the cultivation of plant material is absolutely necessary (LINSKI NS 1975)

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REFERENCES

ASCHER, P D (1966) A gene action model to explain gametophytic self-incompatibility *Euphytica* **15** 179–183

— & S J PELOQUIN (1966a) Effect of floral aging on the growth of compatible and incompatible pollen tubes in Lilium longiflorum *Amer J Bot* **53** 99–102

— & —(1966b) Influence of temperature on incompatible and compatible pollen tube growth in Lilium longiflorum *Can J Genet Cytol* **8** 661 664

BREWBAKER J L & B H KWACK (1963) The essential role of calcium ion in pollen germination and pollen tube growth *Amer J Bot* **50** 859–865

CHEN, C C & P B GIBSON (1973) Effect of temperature on pollen-tube growth in Trifolium repens after cross- and self-pollinations *Crop Science* **13** 563–566

DANE, F & B MELTON (1973) Effect of temperature on self- and cross-compatibility and in vitro pollen growth characteristics in Alfalfa *Crop Science* **13** 587–591

DELBART C B BRIS H F LINSKENS R HINDER & D COUSTAUT (1980) Analysis of glycosphingolipids of Petunia hybrida, a self-incompatible species II Evolution of the fatty acids composition after cross- and self pollination *Proc Kon Ned Akad Wet* **C 83** 241 254

DONK, J A W VAN DER (1974) Differential synthesis of RNA in self- and cross-pollinated styles of Petunia hybrida L *Molec Gen Genet* **131** 1 8

— (1975) Recognition and gene expression during the incompatibility reaction in Petunia hybrida I *Molec gen Genet* **141** 305–316

FURTH A J (1980) *Lipids and polysaccharides* Studies in Biology no 125 Edward Arnold, London

GONAI, H & K HINATA (1971) Effect of temperature on pistil growth and phenotypic expression of self incompatibility in Brassica oleracea L *Jap J Breed* **21** 195–198

HAYASE, H (1955) Effect of temperature before and after anthesis on pollen germinating power in cucumber and egg plant *J Jap Soc Hort Sc* **24** 143–148

HERPEN, M M A VAN (1981) Effect of season age and temperature on the protein pattern of pollen and styles in Petunia hybrida *Acta Bot Neerl* **30**(4) (next issue)

HERRERO M & H G DICKINSON (1980) Ultrastructural and physiological differences between buds and mature flowers of Petunia hybrida prior to and following pollination *Planta* **148** 138 145

KWACK B H (1965) Stylar culture of pollen and physiological studies of self-incompatibility in Oenothera organensis *Physiol Plant* **18** 297–305

LEWIS, D (1942) The physiology of incompatibility in plants I The effect of temperature *Proc Roy Soc* **B 131** 13 26

— (1952) Serological reactions of pollen incompatibility substances *Proc Roy Soc* **B 140** 127 135

LINSKENS H F (1964) The influence of castration on pollen tube growth after self-pollination In *Pollen Physiology and Fertilization* (H F LINSKENS, ed) pp 230 236 Amsterdam North-Holland

— (1973) Reaction of inhibition during incompatible pollination and its elimination *Fiziol Rast* **20** 192–203

— (1975) Incompatibility in Petunia *Proc Roy Soc* **B188** 299 311

— (1977) Incompatibility reactions during the flowering period of several Petunia clones *Acta Bot Neerl* **26** 411 415

— & K ESSER (1957) Über eine spezifische Anfarbung der Pollenschlauche im Griffel und die Zahl der Kallosepropfen nach Selbstung und Fremdung *Naturwissenschaften* **44** 10

STRAUB, J (1958) Das Überwinden der Selbststerilitat *Z Bot* **46** 98–111

TER-AVANESIAN, D V (1978) The effect of varying the number of pollen grains used in fertilization *Theor Appl Genet* **52** 77–79

TOWNSEND C E (1968) Self-compatibility studies with diploid alsike clover, Trifolium hybridum L III Response to temperature *Crop Science* **8** 269–272

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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 PIFRARD 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).

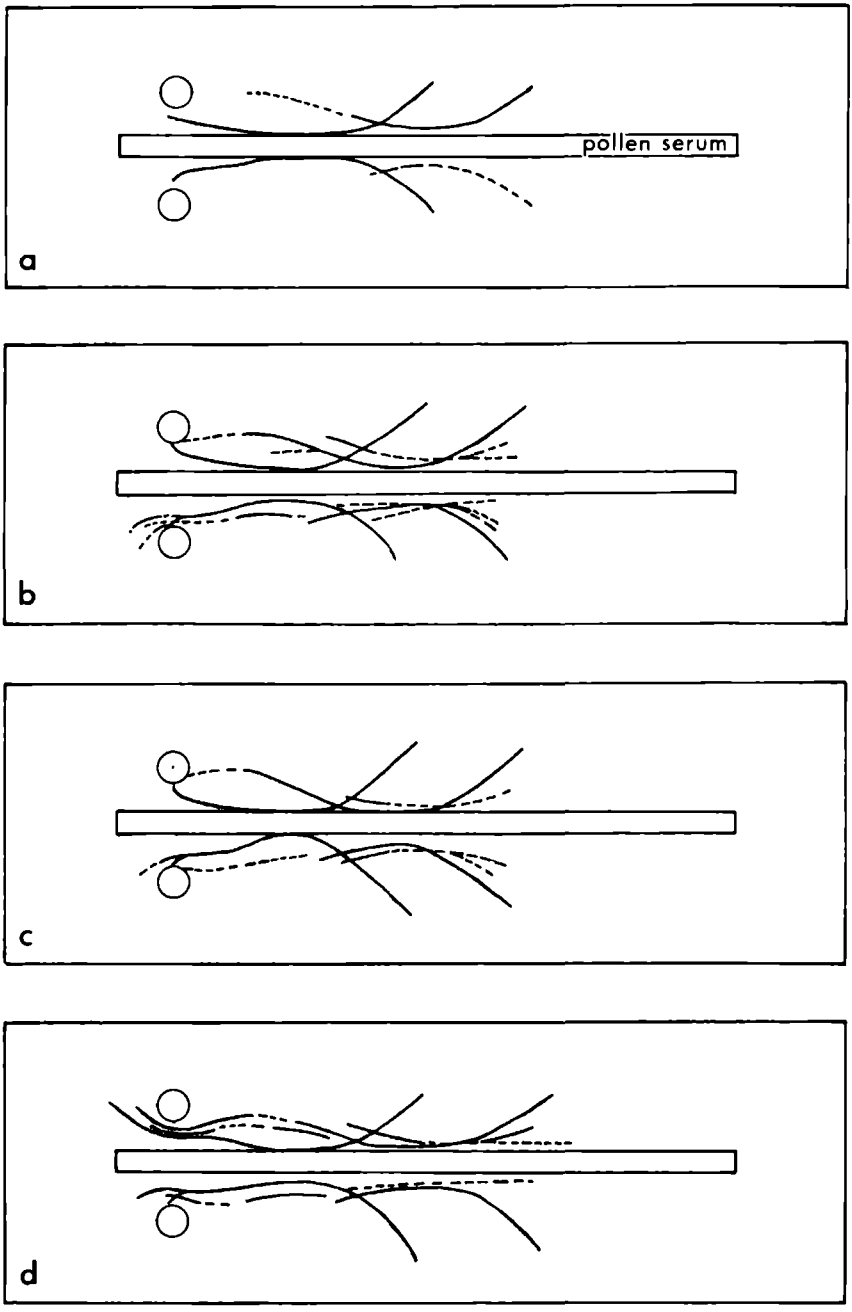

Fig 1 Immunoelectrophoretic patterns of various pollen antigens, from plants developed from winter cuttings, against pollen serum

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

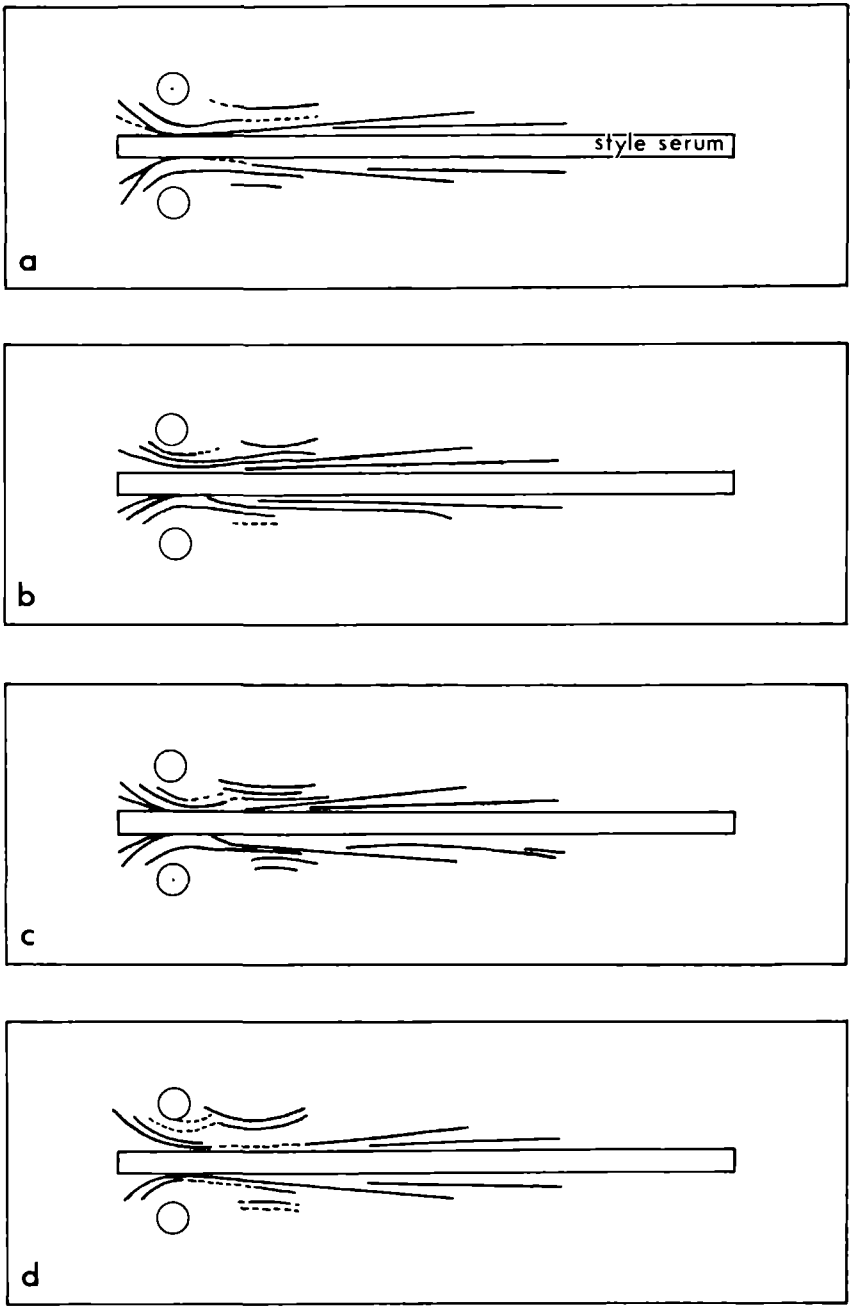

Fig 2 Immunoelectrophoretic patterns of various style antigens, from plants developed from winter cuttings, against style serum

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

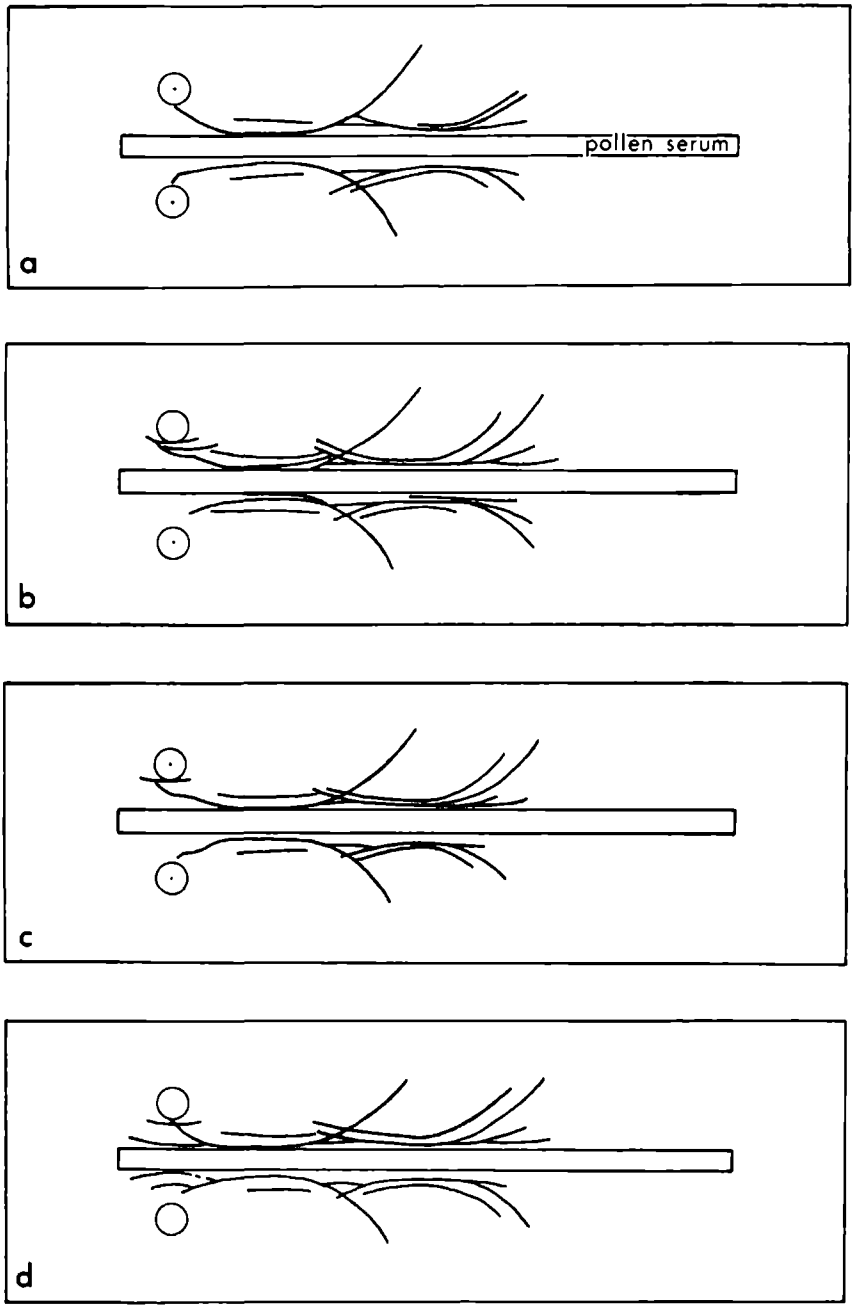

Fig 3 Immunoelectrophoretic patterns of various pollen antigens, from plants developed from summer cuttings, against pollen serum

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

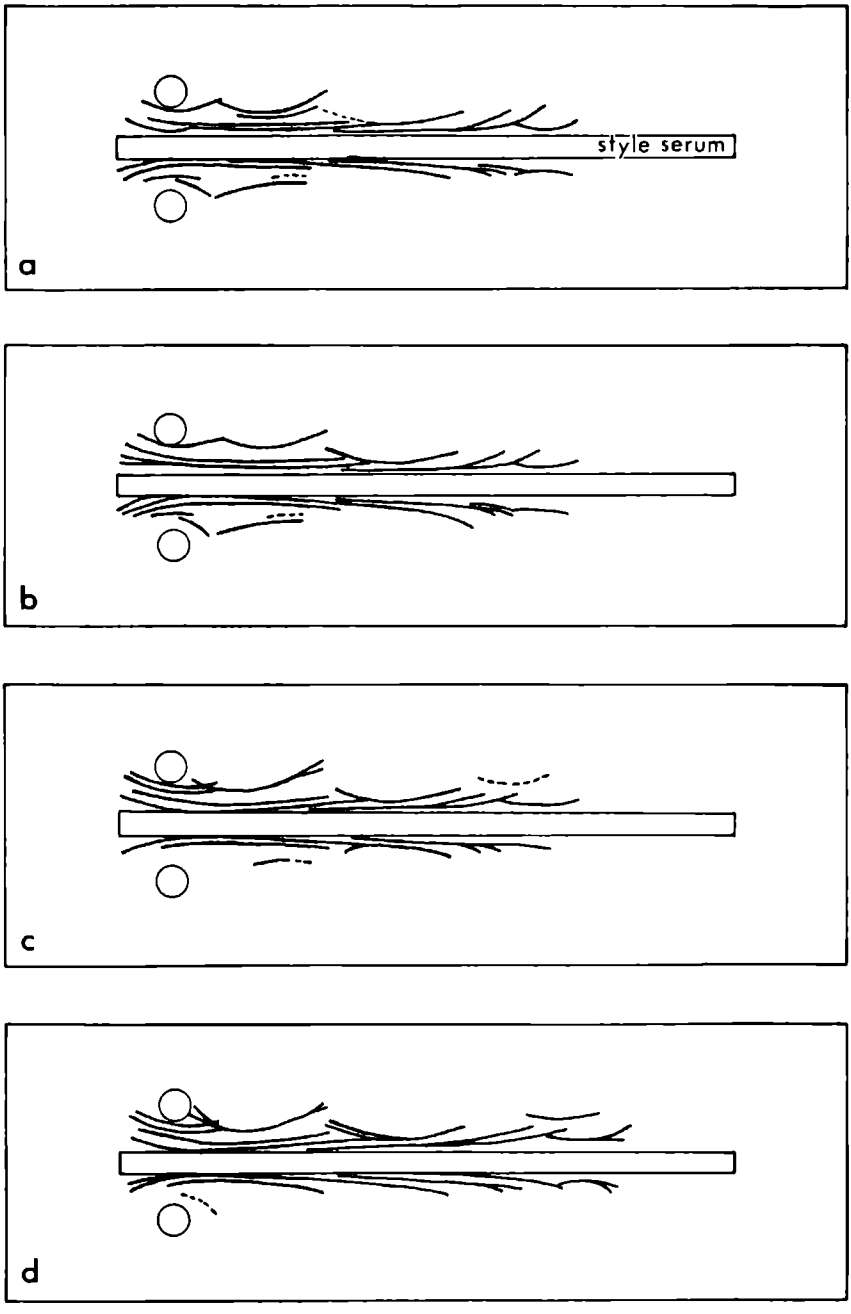

Fig 4 Immunoelectrophoretic patterns of various style antigens, from plants developed from summer cuttings, against style serum

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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REFERENCES

ASCHER, P D (1966) A gene action model to explain gametophytic self-incompatibility *Euphytica* **15** 179–183

— & S J PELOQUIN (1966a) Influence of temperature on incompatible and compatible pollen tube growth in Lilium longiflorum *Can J Genet Cytol* **8** 661 664

—&—(1966b) Effect of floral aging on the growth of compatible and incompatible pollen tubes in Lilium longiflorum *Amer J Bot* **53** 99–102

BREDEMEIJER G M M (1971) *Glutamate dehydrogenase from pollen styles and leaves Properties of purified and non-purified glutamate dehydrogenases from Petunia hybrida* Thesis, University of Nijmegen

DANE F & B MELTON (1973) Effect of temperature on self- and cross-compatibility and in vitro pollen growth characteristics in Alfalfa *Crop Sci* **13** 587–591

DELBART, C, B BRIS, H F LINSKENS R LINDER & D COUSTAUT (1980) Analysis of glycosphingolipids of Petunia hybrida, a self-incompatible species II Evolution of the fatty acids composition after cross- and self-pollination *Proc Kon Ned Akad Wet C* **83** 241 254

DONK J A W VAN DER (1974a) Differential synthesis of RNA in self- and cross-pollinated styles of Petunia hybrida L *Molec Gen Genet* **131** 1-8

— (1974b) Synthesis of RNA and protein as a function of time and type of pollen tube - style interaction in Petunia hybrida L *Molec Gen Genet* **134** 93 98

—(1975) Recognition and gene expression during the incompatibility reaction in Petunia hybrida L *Molec Gen Genet* **141** 305 316

FURTH, A J (1980) *Lipids and polysaccharides* Studies in Biology no 125, Edward Arnold, London

HECHT, A (1964) Partial inactivation of an incompatibility substance in the stigmas and styles of Oenothera In *Pollen Physiology and Fertilization* (H F LINSKENS, ed) pp 237 243 Amsterdam North-Holland Publ Co

HERPEN, M M A VAN & H F LINSKENS (1981) Effect of season, plant age and temperature during plant growth on compatible and incompatible pollen tube growth in Petunia hybrida *Acta Bot Neerl* **30** 209 218

HERRERO M & H G DICKINSON (1980) Ultrastructural and physiological differences between buds and mature flowers of Petunia hybrida prior to and following pollination *Planta* **148** 138 145

HIRATSUKA, S & T TEZUKA (1980) Changes in proteins in pistils after self- and cross-pollination in Japanese pear *J Japan Soc Hort Sci* **49** 57 64

HUDSON, L & F C HAY (1976) *Practical immunology* Blackwell Scientific Publications

KOVALEVA L V, E L MILYAEVA & M K H CHAILAKHYAN (1978) Overcoming self-incompatibility by inhibitors of nucleic acid and protein metabolism *Phytomorphology* **28** 445 449

KWACK B H (1965) Stylar culture of pollen and physiological studies of self-incompatibility in Oenothera organensis *Physiol Plant* **18** 297 305

LEWIS, D (1942) The physiology of incompatibility in plants I The effect of temperature *Proc Roy Soc Lond* **B 131** 13 26

LINSKENS, H F (1955) Physiologische Untersuchungen der Pollenschlauch-Hemmung selbststeriler Petunien *Z Bot* **43** 1 44

— (1964) The influence of castration on pollen tube growth after self-pollination In *Pollen Physiology and Fertilization* (H F LINSKENS, ed), pp 230 236 Amsterdam North-Holland Publ Co

—(1966) Die Änderung des Protein- und Enzym-Musters während der Pollenmeiose und Pollenentwicklung *Planta* **69** 79–91

— (1973) Reaction of inhibition during incompatible pollination and its elimination *Fiziol Rast* **20** 192 203

— (1975) Incompatibility in Petunia *Proc Roy Soc* **B 188** 299–311

— (1977) Incompatibility reactions during the flowering period of several Petunia clones *Acta Bot Neerl* **26** 411 415

— & J TUPY (1966) The amino acids pool in the style of self incompatible strains of Petunia after self- and cross-pollination *Genet Breed Res* **36** 151 158

—, R HAVEZ, R LINDER, M SALDEN, A RANDOUX, D LANIEZ & D COUSTAUT (1969) Etude des glycanne-hydrolases au cours de la croissance du pollen chez Petunia hybrida auto-incompatible *C R Acad Sci (Paris)* **269** 1855–1857

—, —, —, —, —, — & — (1970) Etude des glycoproteines et glycannes-hydrolases au cours de la pollinisation chez Petunia hybrida auto-incompatible *Bull Soc Pharmacie France du Nord* **1** 1 16

LOWRY O H, N J ROSEBROUGH A L FARR & R J RANDALL (1951) Protein measurement with the Folin phenol reagent *J Biol Chem* **193** 265 275

PANDEY, K K (1972) Isozyme specificity to temperature *Nature New Biology* **239** 27–29

PIERARD, D, A JACQMARD & G BERNIER (1979) Changements de la composition en proteines des differentes parties du bourgeon apical de Sinapis alba au cours de sa mise a fleurs *C R Acad Sci (Paris)* **289** 761 763

ROGGEN, H P J R (1967) Changes in enzyme activities during the progamic phase in Petunia hybrida *Acta Bot Neerl* **16** 1 31

STRAUB, J (1958) Das Überwinden der Selbststerilitat *Z Bot* **46** 98 111

TOWNSEND, C E (1968) Self-compatibility studies with diploid alsike clover, Trifolium hybridum L III Response to temperature *Crop Science* **8** 269 272

THE EFFECT OF TEMPERATURE ON THE ULTRASTRUCTURE OF TRANSMITTING TISSUE IN THE STYLE AND ON THE FREE CARBOHYDRATE CONTENT IN POLLEN AND STYLES OF PETUNIA HYBRIDA

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SUMMARY

Styles developed at a high temperature day/night regime of 25.5/18°C ("warm styles") contain more intercellular substance than styles developed at a low temperature day/night regime of 19.5/18°C ("cold styles"). In "warm styles" the intercellular substance increases with increasing age of the plant. The total amount of free carbohydrates (low molecular weight carbohydrates) is highest in "cold styles". In "warm styles" the free carbohydrate content decreases with increasing age of the plant. Pollen developed at the high day/night regime of 25.5/18°C ("warm pollen") have more free carbohydrates than pollen developed at the low temperature regime of 19.5/18°C ("cold pollen").

A supplement of 24 h of the high temperature regime given to flowers developed at the low temperature regime had no effect on the free carbohydrate content of the pollen, but did influence the free carbohydrate content in the style of the same flower.

1.INTRODUCTION

E.M. studies of unpollinated (KROH & VAN BAKEL 1973) and pollinated (VAN DER PLUIJM & LINSKENS 1966) styles of *Petunia hybrida* showed that the cells of the transmitting tissue (TT) are surrounded by an intercellular substance (IS), a mixture of mainly acidic carbohydrates (KROH 1973) which can be considered as a secretion product of the TT and compared with the mucilage that fills the canal of open styles (SASSEN 1974).

Labelling experiments (LINSKENS & ESSER 1959), E.M. studies (VAN DER PLUIJM & LINSKENS 1966) and biochemical analyses (LINSKENS 1955) have indicated that the pollen tubes take up material, such as free carbohydrates (LINSKENS 1955), from the style during their growth through the IS.

The length of compatible pollen tubes is influenced by the temperature during stylar development (VAN HERPEN & LINSKENS 1981). The question arises whether or not the temperature during stylar development has an effect on the TT, which is the normal environment for growing pollen tubes and influences the pollen tube length, as well as on the amount of free carbohydrates in the style.

2.MATERIALS AND METHODS

T_2U summer-cuttings were grown in two plant growth chambers with day/night regimes at 19.5/18°C and 25.5/18°C, respectively as described previously (VAN HERPEN & LINSKENS 1981).

The styles developed at different temperatures were harvested at 9.00 a.m. from flowers which had not yet opened. Pieces of 1 to 2 mm were cut from the middle of the styles, fixed in 6.25% glutaraldehyde in 0.2 M cacodylate buffer pH 7.2 and kept for 2 h at room temperature. After rinsing in the same buffer style pieces were post-fixed in 2% in OsO_4 for 2 h, dehydrated in a graded ethanol series and embedded in Spurr's plastic. Sections were stained with uranylacetate and leadcitrate and examined with a Philips E.M. 201. For the determination of free carbohydrates anthers and styles were collected from flower buds just before anthesis. The anthers were dried at 23°C for 24 h in the dark and 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 (VAN HERPEN 1981). Pollen and pulverized styles were homogenized in 80% ethanol using a Braun Potter homogenizer (30 strokes at 700 r.p.m.); after centrifugation (15,000 × g) the supernatant was passed through a Dowex 50 × 8 (H^+) column.

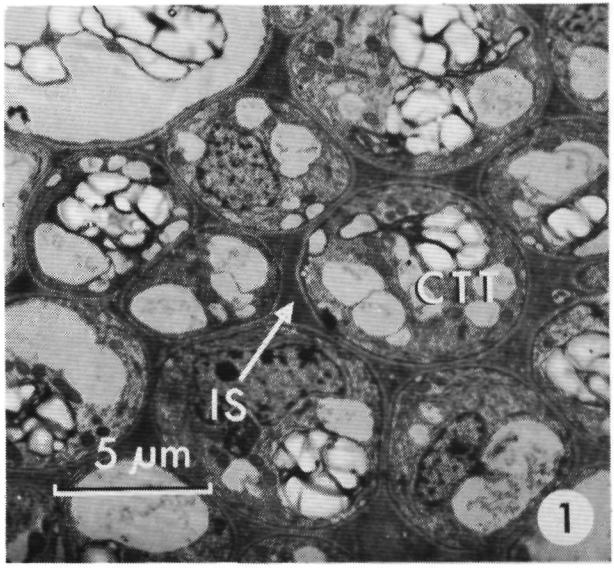
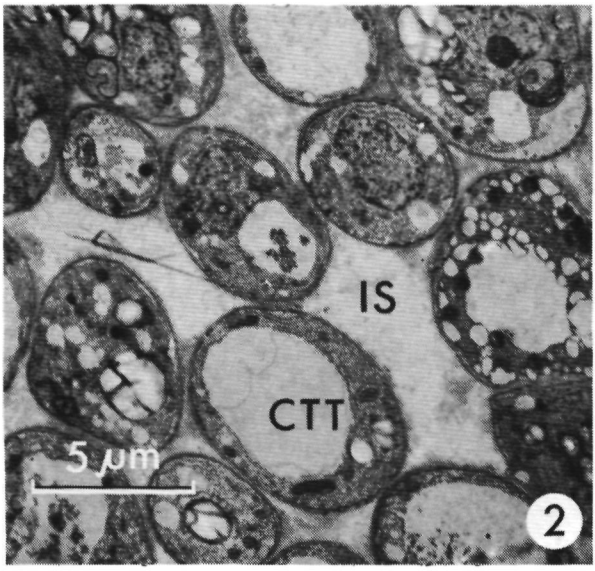


Fig.1. Transverse section through the stylar transmitting tissue of a "cold style" (plants 14 or 18 weeks old) whether or not supplemented with 24 h of the high temperature regime of 25.5/18°C. CTT = Cell of transmitting tissue. IS = Intercellular substance.



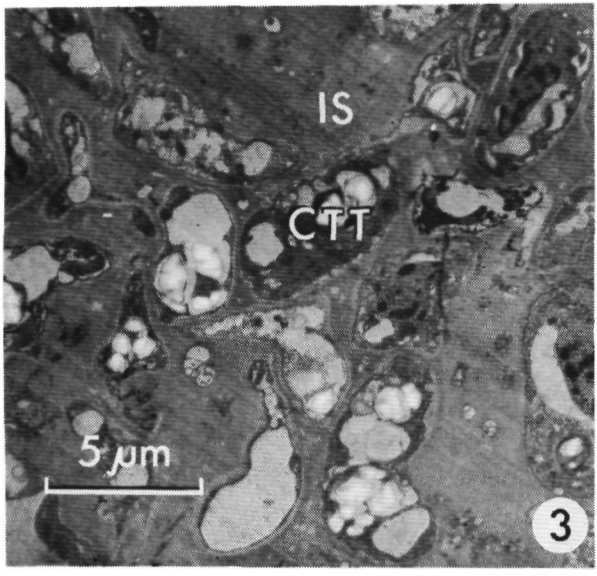


Fig.2. Transverse section through the transmitting tissue of a "warm style", from a 14 week-old plant.
Fig.3. Transverse section through the transmitting tissue of a "warm style", from a 18 week-old plant.
CTT = Cell of transmitting tissue. IS = Intercellular substance.

The concentration of free carbohydrates in the effluent was measured by the method of DUBOIS *et al.*(1956).

3. RESULTS

Styles developed at the high temperature regime of 25.5/18°C ("warm styles") have more IS (*figs.1, 2*) and less free carbohydrates (*table 1*) than styles developed at the low regime of 19.5/18 C ("cold styles") whether or not supplemented with 24 h of the high temperature regime (*fig.1, table 1*); only in "warm styles" an increase in IS and a decrease in dry weight with increasing age of the plant is observed (*fig.3, table 1*).

The free carbohydrate content, in styles developed at the low temperature regime, was disturbed for a long time when those styles were pretreated with
24 h of the high temperature regime (*fig.4*); no such effect could be detected in pollen developed at the low temperature regime supplemented with 24 h of the high temperature regime (*table 2*). Pollen developed at high temperature conditions ("warm pollen") has more free carbohydrates than pollen developed under the low temperature condition ("cold pollen") as can be seen in *table 2.*

Table 1. Effect of temperature and age of the plant on dry weight and free carbohydrate content of styles. Average of four analyses on 60 styles each, with standard deviation.

Temp. regime during style development	Age plant (weeks)	Dry weight per style(mg)	Free carbohydrates per style(μg)
19.5/18°C	14	1.54 ± 0.02	214.7 ± 5.0
25.5/18°C	14	1.55 ± 0.03	181.8 ± 9.2
19.5/18°C	18	1.54 ± 0.02	209.2 ± 3.2
19.5/18°C + 24 h 25.5/18°C	18	1.56 ± 0.03	210.4 ± 4.8
25.5/18°C	18	1.39 ± 0.02	163.4 ± 8.0

Table 2. Effect of temperature and age of the plant on the free carbohydrate content of pollen. Average of four analyses on 25 mg pollen, with standard deviation.

Temp. regime during pollen development	Age plant (weeks)	Free carbohydrates(μg) per mg pollen
19.5/18°C	14	156.8 ± 7.5
25.5/18°C	14	183.2 ± 5.2
19.5/18°C	18	159.5 ± 4.2
19.5/18°C + 24 h 25.5/18°C	18	151.6 ± 3.5
25.5/18°C	18	193.2 ± 9.7

4. DISCUSSION

Carbohydrates play a role in the metabolism of the plant as energy carriers, as reserves and, above all, as building materials (PISEK *et al.* 1973).

The temperature during stylar development influences the mass of IS (*figs.1, 2, 3*), the free carbohydrate content of styles (*tables 1, 2*) as well as the length of pollen tubes after compatible pollination of those styles (VAN HERPEN & LINSKENS 1981). It is therefore possible that the length of the pollen tubes resulting from a compatible pollination is either determined by the mass of IS or the amount of available free carbohydrates in the pollinated style. In styles developed at the low temperature regime, whether or not supplemented with 24 h of the high temperature regime, the IS is not different. However, the free carbohydrate content in the styles treated with 24 h of the high temperature regime oscillates with time (*fig.4*). The fact that 'cross'-pollen tube growth is enhanced by the temperature treatment of the style (VAN HERPEN & LINSKENS 1981) and is not influenced by the fluctuation of the free carbohydrate content in that same style, together with the observations that the free carbohydrate content in the styles developed at the low temperature regime is highest and the pollen tube length lowest (VAN HERPEN & LINSKENS 1981) compared to styles developed at the high temperature regime, justifies the conclusion that the length of the compatible pollen tubes is not correlated with the free carbohydrate content of the style. This does not exclude the uptake of free carbohydrates by the growing pollen tubes, but indicates that the amount available does not constitute a limiting factor. This leads to the remaining possibility that the mass of IS determines the length of the pollen tubes.

KROH & HELSPER (1974) could not establish whether the IS in the stylar TT is first broken down to monosaccharides and then incorporated into the growing pollen tubes or is first taken up partly unchanged and subsequently broken down. The conclusions stated above, support the latter possibility.

LINSKENS (1975) stated that the style has to switch on or to activate additional pollen enzyme system(s) which prepare the way towards the ovary through the IS. Such a switch on or activation does not occur after an incompatible pollination and could therefore explain why the incompatible pollen tube length is determined by the temperature at which the pollen developed (VAN HERPEN & LINSKENS 1981). The temperature during pollen development (BRINK 1924) has its effect on the protein (VAN HERPEN 1981) as well as on the free carbohydrate content (see results) of that pollen, and either one or both, could be responsible for the longer incompatible pollen tube length.

In the case of a compatible pollination the switch on or activation can be successful and the length of the tubes depends on the temperature during style development (VAN HERPEN & LINSKENS 1981). The higher

the temperature during style development the more free carbohydrates are probably polymerised into IS resulting in a decrease of free carbohydrates (*table 1*)and correspondent increase of IS (*figs.1, 2*) resulting in the above stated positive effect on the length of the compatible pollen tubes growing through it. This seems to be confirmed by the following: the lower the temperature to which the plant is exposed the more pronounced is the rise in sugar concentration at the expense of the stored carbohydrates, particularly oligosaccharides; high temperatures lead to sugar-starch conversions (PISEK *et al.* 1973).

The age of the plant has an effect on the amount of IS in "warm styles" (*figs.2, 3*) but not on the length of compatible pollen tubes (VAN HERPEN & LINSKENS 1981). This can be explained when the effectiveness of the pollen enzyme system described by LINSKENS (1975) is determined by the temperature during the progamic phase.

The results of this paper, together with those reported previously, indicate that the compatible pollen tube length is correlated with the mass of IS, which is determined by the temperature during style development, and the effectiveness of the pollen enzyme system itself, which in turn depends on the temperature during the progamic phase. The incompatible pollen tube length is correlated with the properties of the pollen itself, influenced by the temperature during pollen development and the temperature during the progamic phase.

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REFERENCES

DUBOIS, M., K.A.GILLES, J.K.HAMILTON, P.A.REBERS & R.SMITH (1956): Colorimetric method for determination of sugars and related substances. *Analyt. Chem* **28**:350-356.

HERPEN, M.M.A.VAN (1981): Effect of season, age and temperature on the protein pattern of pollen and styles in Petunia hybrida. *Acta Bot. Neerl.* **30**:277-287.

— & H.F.LINSKENS (1981): Effect of season, plant age and temperature during plant growth on compatible and incompatible pollen tube growth in Petunia hybrida. *Acta Bot. Neerl.* **30**:209-218.

KROH, M (1973) Stylar transmitting tissue In Biogenesis of plant cell wall polysaccharides Pp 195-205 Ed F LOEWUS Acad Press, New York
— & C H J VAN BAKEL (1973) Incorporation of label into the intercellular substance of stylar transmitting tissue from Petunia pistils labeled with tritiated myo-inositol An electron microscopic autoradiographic study *Acta Bot Neerl* **22** 106-111
— & J P F G HELSPER (1974) Transmitting tissue and pollen tube growth In Fertilization in higher plants Ed H F LINSKENS North-Holland, Amsterdam Pp 167-175
LINSKENS, H F (1955) Physiologische Untersuchungen der Pollenschlauch-Hemmung selbststeriler Petunien *Z Bot* **42** 1-44
— (1975) Incompatibility in Petunia *Proc R Soc (London)* **B188** 299-311
— & K ESSER (1959) Stoffaufnahme der Pollenschlauche aus dem Leitgewebe des Griffels *Proc Kon Ned Acad Wetens (Amsterdam)* **C62** 150-154
PISEK, A , W LARCHER, A VEGIS & K NAPP-ZINN (1983) The normal temperature range In Temperature and life Ed H PRECHT, J CHRISTOPHERSEN, H HENSEL & W LARCHER Springer-Verlag Berlin-Heidelberg-New York, pp 102-194
PLUIJM, J VAN DER & H F LINSKENS (1966) Feinstruktur der Pollenschlauche im Griffel von Petunia *Zuchter* **36** 220-224
SASSEN, M M A VAN (1984) The stylar transmitting tissue *Acta Bot Neerl* **23** 99-108

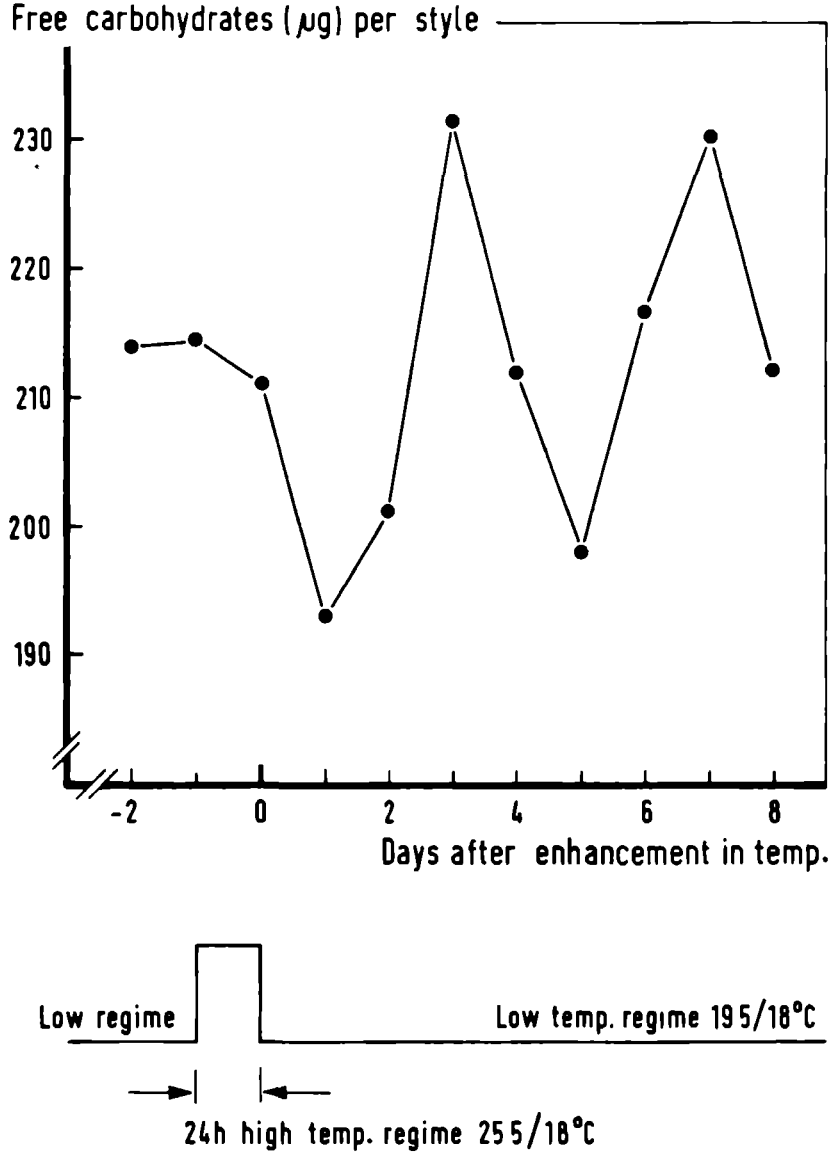

Fig 4 Effect of 24h of the high temperature regime 25 5/18°C on the free carbohydrate content in styles developed at the low temperature regime 19 5/18°C

PHYTIC ACID CONTENT OF POLLEN DEVELOPED AT DIFFERENT TEMPERATURES

SUMMARY

Pollen developed at the high temperature regime of 25.5/18°C has significantly more phytic acid than pollen developed at the low temperature regime of 19.5/18°C.

1. INTRODUCTION

There is support for the hypothesis that phytic acid in pollen is essential for tube elongation because it is needed as a reserve for myo-inositol, which is used for uronic acid formation during pollen tube wall synthesis (JACKSON *et al.* 1982, JACKSON & LINSKENS 1982).

T_2U (S_3S_3) pollen developed at the high temperature regime of 25.5/18°C make a longer pollen tube than S_3S_3 pollen developed at the lower temperature regime of 19.5/18°C (VAN HERPEN & LINSKENS 1981). So there is good reason to investigate the possibility that the length of the pollen tubes of pollen developed at different temperatures can either be correlated with the total content of phytic acid in that pollen or a different metabolic activity of that pollen after germination.

2. MATERIALS AND METHODS

Pollen was collected from the homozygous clone T_2U (S_3S_3)grown, from the moment of cutting, in two plant growth chambers with day/night regimes at 19.5/18°C and 25.5/18°C, respectively as described previously (VAN HERPEN & LINSKENS 1981). Phytic acid was extracted from the pollen with 0.02 M Na_4EDTA solution and determined by paper electrophoresis (JACKSON *et al.* 1982).

3. RESULTS

Pollen developed at the high temperature regime of 25.5/18°C and the low temperature regime of 19.5/18°C has 33.13 and 23.75 nmol, respectively phytic acid per mg pollen. The metabolic activity of the pollen after germination was studied in collaboration with J.F. JACKSON, Adelaide (Australia) and the preliminary results are incorporated in the "Discussion section".

4. DISCUSSION

The fact that the phytic acid reserve in pollen depends on the temperature during pollen development is in agreement with the statement of BRINK (1924) that the nature of reserve material is dependent to a certain extent upon seasonal conditions.

As far as I know now, temperature during pollen development determines the content of free carbohydrates (VAN HERPEN 1983), phytic acid (see results) and total protein (VAN HERPEN 1981) and also induces a change in the metabolic activity of the pollen after germination: pollen developed under the high temperature regime do synthesize, in the first hours after germination *in vitro*, proteins (at least six proteins) in larger amounts than pollen developed under the lower regime (J.F.JACKSON, personal communication).

The greater length of the pollen tubes from pollen developed at the higher temperature regime may be a consequence of the higher content of phytic acid and the higher metabolic activity in the pollen after pollination, the latter resulting in more proteins as, for example, phytase which catalyses rapid degradation of phytic acid.

ACKNOWLEDGEMENTS

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REFERENCES

BRINK, R.A. (1924): The physiology of pollen. I The requirements for growth. *Am. J. Bot.* **11**:218-228.

HERPEN, M.M.A.VAN (1981): Effect of season, age and temperature on the protein pattern of pollen and styles in Petunia hybrida. *Acta Bot. Neerl.* **30**:277-287.

— & H.F.LINSKENS (1981): Effect of season, plant age and temperature during plant growth on compatible and incompatible pollen tube growth in Petunia hybrida. *Acta Bot. Neerl.* **30**:209-218.

— (1983): Temperature, Petunia stylar ultrastructure and free carbohydrates. *Proceedings 7th International Symposium on Fertilization and Embryogenesis in Ovulated Plants.* 14-17th June 1982. Račková Dolina (Czechoslovakia). Pp.227-230.

JACKSON, J.F.& H.F.LINSKENS (1982): Phytic acid in Petunia hybrida pollen is hydrolysed during germination by a phytase. *Acta Bot. Neerl.* **31**:441-447.

—, G.P.JONES & H.F.LINSKENS (1982): Phytic acid in pollen. *Phytochemistry* **21**:1255-1258.

Acta Bot Neerl 33(2), May 1984, p 195–203

EXTRACTS FROM STYLES, DEVELOPED AT DIFFERENT TEMPERATURES, AND THEIR EFFECT ON COMPATIBILITY OF PETUNIA HYBRIDA IN EXCISED-STYLE CULTURE

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SUMMARY

The growth of *Petunia* clone W166K (S_1S_2, self-incompatible) pollen tubes in Brewbaker & Kwack's medium is inhibited by extracts from clone T_2U (S_3S_3, also self-incompatible) styles The styles were collected one day before anthesis from plants grown either at a day/night temperature regime of 25 5/18°C (extracts A) or 19 5°/18°C (extracts B) High molecular (protein) extracts B had a much stronger effect than high molecular extracts A After enzymatic breakdown of the proteins the inhibition by extract A was as strong as that by extract B. From these results it is concluded that prior to anthesis pollen tube growth stimulating proteins are being synthesized in the style when the day temperature is sufficiently high

The inhibitory effects of the low molecular weight carbohydrate extracts A and B were the same.

1. INTRODUCTION

Temperature during style development has an effect on the length of pollen tubes after a compatible pollination: the length of those tubes is greater when the styles developed under the 25.5/18°C temperature regime instead of under the lower temperature regime of 19.5/18°C (VAN HERPEN & LINSKENS 1981).

There is no difference in total protein content between unpollinated styles either developed under the high or low temperature regime, but the immunologically determined protein patterns of those styles differ significantly (VAN HERPEN 1981).

So the question arises whether or not polypeptides, synthesized before pollination could have an impact on the length of pollen tubes after compatible pollinations. According to the model of VAN DER DONK (1975) the question seems rather irrelevant, because the stylar polypeptides do have an influence on the tube length in a compatible situation, but their synthesis is only restricted to the progamic phase.

Labelling experiments (LINSKENS & ESSER 1959), E.M. studies (VAN DER PLUIJM & LINSKENS 1966) and biochemical analysis (LINSKENS 1955) have indicated that pollen tubes take up material, such as free sugars from the style during

their growth through the intercellular substance (I.S.). The length of the pollen tubes resulting from a compatible pollination may be determined by the mass of intercellular substance (I.S.) or by the amount of available free carbohydrates in the style; unless of course the I.S. is completely broken down to free carbohydrates by the growing pollen tube, the total amount of free carbohydrates could not have an influence on the pollen tube length, because the measured free carbohydrate content in unpollinated styles developed at the low temperature regime is the highest and the pollen tube length in those styles the least compared to the styles developed at the high temperature regime (VAN HERPEN & LINSKENS 1981; VAN HERPEN, 1983).

With the help of the excised style technique it should be possible to determine whether or not proteins and free carbohydrates have an influence on the length of the compatible pollen tubes.

Two methods were available: the 'improved excised style culture" from NIIMI (1982) and the "semi-vitro culture" (SCHOCH-BODMER 1932; STRAUB 1946; BREWBAKER & MAJUMDER 1961).

2. MATERIALS AND METHODS

2.1. Plant Material

Two clones of self-incompatible *Petunia hybrida* were used: T_2U (S_1S_3) as style, and W166K (S_1S_2) as pollen provider. The T_2U summer-cuttings were grown in two plant growth chambers with day/night regimes at 19.5/18 C and 25.5/18 C respectively, as described previously (VAN HERPEN & LINSKENS 1981). W166K was grown in the greenhouse, because temperature during pollen development had no effect on the tube length of that pollen (VAN HERPEN & LINSKENS 1981). Only cross-pollinations, W166K (S_1S_2) pollen on T_2U (S_3S_3) styles, were performed.

2.2 Collection and storage of pollen, and pollination

Anthers were collected from W166K 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 afterwards stored at −70 C. To avoid self-pollination the T_2U flowers were emasculated, and then pollinated with the stored W166K pollen. Only T_2U styles, developed at the temperature regime of 19.5/18 C, were used for these pollination experiments.

2.3. Pollen tube growth in vivo and vitro

Two methods were available: "the improved excised style culture" (NIIMI 1982) and the "semi-vitro culture" (SCHOCH-BODMER 1932; STRAUB 1946; BREWBAKER & MAJUMDER 1961). Both were tested and the last one was improved in such a way that it became a good tool for studying the effect of style extracts on pollen tube growth. The pollen germination and subsequent pollen tube growth were allowed for exactly 4 hours on the plant at a temperature of 19.5 ± 0.2 C.

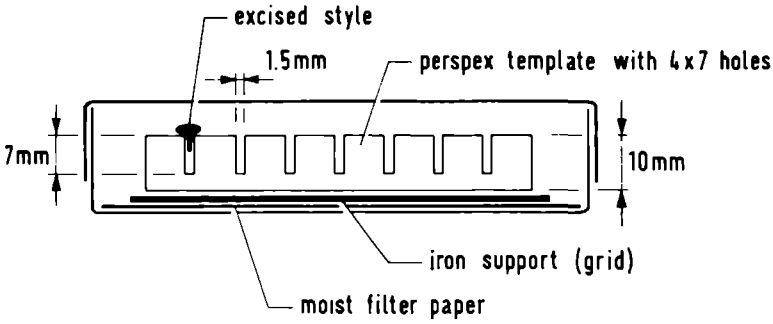


Fig. 1. Apparatus for excised style culture.

Seven flowers (pollinated) were collected at random and the styles were cut with a sharp, scoured, razor blade 5 mm from the stigma tip. These excised-styles were put into holes filled with 10 µl of medium (pollen culture medium according to SHARMA & SHIVANNA 1982 or BREWBAKER & KWACK 1963) with or without style extracts. The perspex template with 4 × 7 holes was placed on a grid into a water saturated petri-dish (*fig. 1*). The pollen tubes were allowed to grow into the medium for 20 hours at 25 C in the dark.

2.4. Determination of pollen tube length

The excised styles and protruding pollen tubes were straightened on a microscope slide and placed under a light microscope with stage micrometer. The length of the protruding pollen tubes was measured from the cut end of the excised style to the point reached by 90% of the pollen tubes.

2.5. Medium

Two media were tested: BREWBAKER & KWACK medium (1963), adjusted to pH 5.6–5.7 with 0.1 N NaOH or 0.1 N HCl before addition of 10% sucrose, was much better suited for our plant material than the pollen culture medium of SHARMA & SHIVANNA (1982) which had a strong inhibitory effect on the length of the protruding pollen tubes, reducing the length almost to nil. The Brewbaker & Kwack medium was used as "the standard medium" for all experiments.

Table 1. Length in mm of W166K pollen tubes, protruding from cut-ends of excised styles and grown in low molecular carbohydrate extracts of styles developed either at the low or high temperature regime. Low molecular carbohydrate extracts are dissolved in Brewbaker & Kwack medium. Average of 28 observations ± standard error of the mean.

conc. of extract (number of styles per 10 µl medium)	0	0 01	0 03	0 05	0 1
19 5/18 C	3.15 ± 0 15	2.80 ± 0.16	2.70 ± 0.15	2 20 ± 0.12	1 25 ± 0.09
25.5 18 C	3 15 ± 0.15	2.90 ± 0 13	2.50 ± 0.10	2 30 ± 0.20	1.20 ± 0 11

Table 2. Length in mm of W166 pollen tubes, protruding from cut-ends of excised styles and grown in differentially treated extracts of styles developed either at the low or high temperature regime Average of 28 observations ± standard error of the mean.

	Extract 5′ at 25° C	Extract 5′ at 70 C	Extract 5′ at 100 °C
25.5/18°C extract	2.17 ± 0 10	3.04 ± 0.14	3.08 ± 0 15
19.5/18°C extract	1.90 ± 0.12	2 06 ± 0 13	3 07 ± 0.15
	Extract 5′ at 70 C	(Extract + Protease K) 30′ at 25°C, inactivated for 5′ at 70 C	(Extract + Protease K) immediately inactivated for 5′ at 70 C
25.5/18°C extract	3.04 ± 0 14	1 71 ± 0.10	2 60 ± 0.12
19.5/18°C extract	2.06 ± 0.13	1 91 ± 0.10	2.04 ± 0 10
	Extract 5′ at 70°C	(Extract + DNA'se) 30′ at 25°C, inactivated for 5′ at 70°C	(Extract + DNA'se) immediately inactivated for 5′ at 70 C
25.5/18°C extrat	3 04 ± 0.14	2.61 ± 0.09	2.48 ± 0.11
19.5/18°C extract	2 06 ± 0.13	2.63 ± 0.10	2 44 ± 0.08
	Extract 5′ at 100°C	(Extract + RNA'se) 30′ at 25°C, inactivated for 5′ at 100°C	(Extract + RNA'se) immediately inactivated for 5′ at 70°C
25.5/18°C extract	3.08 ± 0.15	2.78 ± 0.11	2.64 ± 0 12
19.5/18°C extract	3.07 ± 0.15	2 71 ± 0.10	2.74 ± 0.10

2.6. Preparation of extracts

High molecular substances: Unpollinated T_2U styles developed under the low or the high temperature regime were collected one day before anthesis, immediately frozen in liquid nitrogen, pulverized and homogenized in 0.1 M Tris-HCl pH 7.2 using a Braun Potter homogenizer (30 strokes at 700 r.p.m.); after centrifugation (15,000 × g) the layer of lipids was removed, and the supernatant was passed through a G-25 column, equilibrated with BREWBAKER & KWACK medium (1963); only the high molecular fractions with more than 25 µg protein per 100 µl (protein determination according to LOWRY et al. 1951) were collected and used for further experiments. The concentration of the extract is presented as the number of styles per 10 µl of the "standard medium".

Low molecular carbohydrates: As described above, with the exception that the styles were homogenized in 80% ethanol and the supernatant was passed through a Dowex 50 × 8 (H^+) column. The concentration of the free sugars in the effluent of the column was measured by the method of DUBOIS et al. (1956). After vacuum-evaporation of the ethanol the free carbohydrate-residue is dissolved in "standard medium" and ready to be used for further experiments. The concentration of the extract was expressed as the number of styles per 10 µl of "standard medium".

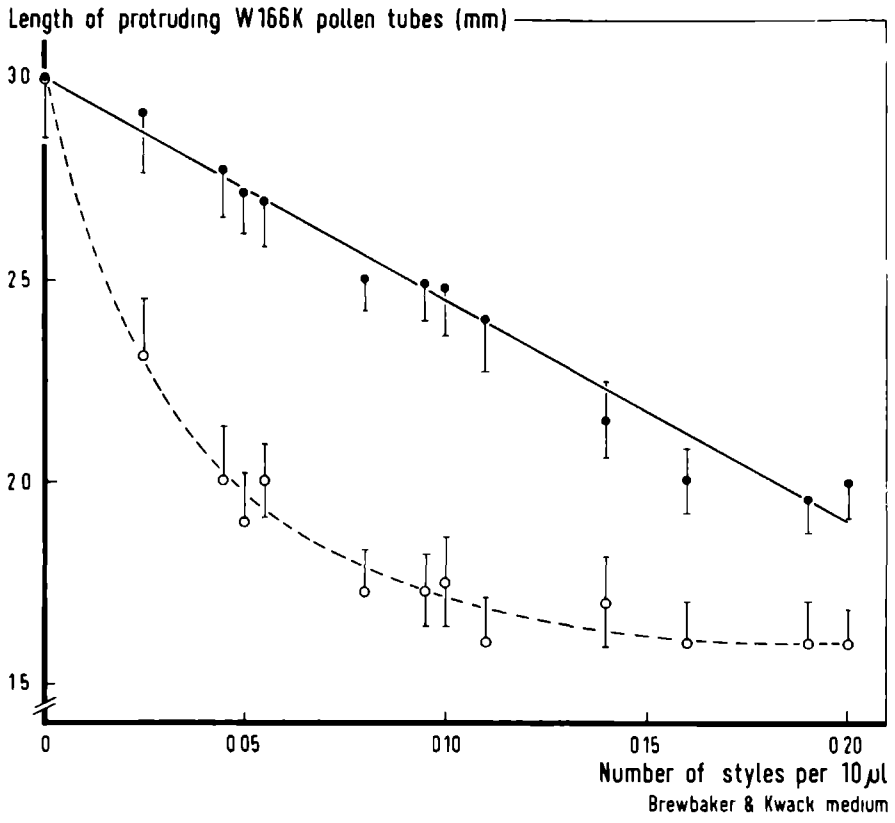


Fig 2 Length of W166K (S_1S_2) pollen tubes grown on the plant for 4 hours at 19 5 C then 20 hours at 25 0 C in excised style culture with T_2U (S_3S_3) extracts of styles developed at 25 5 18 C (•) or 19 5/18 C (○) Average of 28 observations ± standard error of the mean

2 7 Enzymes

Protease K, DNA'se and RNA'se are used in final concentrations of 100 µg/ml Protease K is added to the high molecular extract for 30′ at 25°C and inactivated by a temperature of 70 C for 5′ RNA'se (KUNITZ 1940) and DNA'se are applied for 30′ at 25°C and inactivated respectively at 100°C and 70°C for 5′

3 RESULTS

Methods The disadvantage of the "improved excised style culture" from NIIMI (1982) was that the pollen tubes growing in and on the agar were thin and did not make uniform contact with the extracts dissolved in it The negative point of the "semi-vitro culture" (SCHOCK-BODMER 1932, STRAUB 1946, BREWBAKER & MAJUMDER 1961) was its difficulty to prepare and incubate a large number of excised styles respectively in a short time over a long period of time Improve-

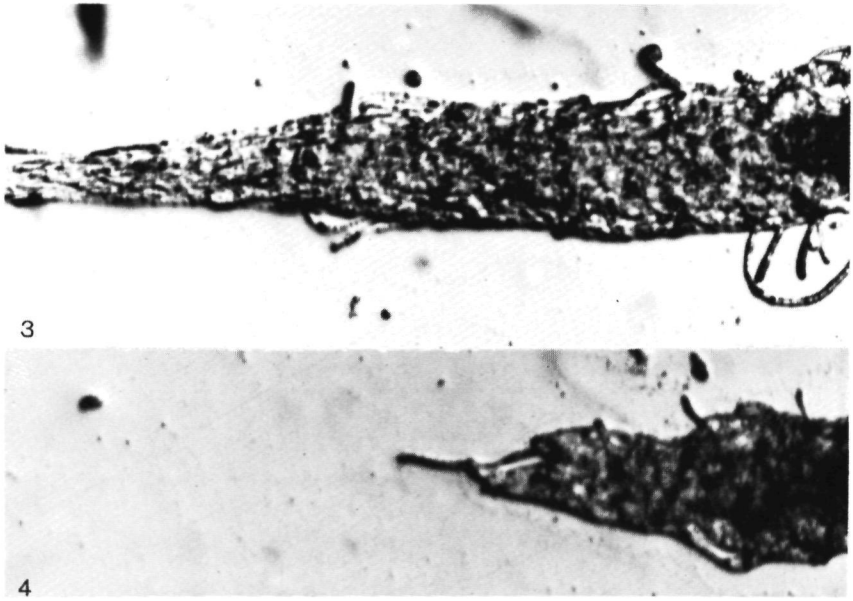


Fig. 3. Protruding pollen tubes growing in extracts of styles developed at the high temperatue regime of 25.5/18°C.
Fig. 4. Protruding pollen tubes growing either in extracts of styles developed at the low temperature regime of 19.5/18°C or in extracts of styles developed at 25.5/18°C and treated with protease K.

ment of the last method (see Materials and Methods 2.3) finally made it a good technique for our purpose.

Low molecular carbohydrate extracts: No differences in pollen tube length could be detected between pollen tubes growing in the low molecular carbohydrate extracts of unpollinated styles developed either at the low (19.5/18°C) or the high temperature (25.5/18°C) regime (*table 1*).

High molecular extracts: The length of the protruding pollen tubes depends on whether the extract is made of styles developed at the high or low temperature regime, and on the concentration of the extract (*fig. 2*). The difference between the style 25.5/18°C and the 19.5/18°C extract is the most when the concentration of the extracts equals 0.05 style per 10 µl standard medium of BREWBAKER & KWACK (*figs. 2, 3* and *4*). This particular concentration will be used in further experiments to determine which compound, in the high molecular extract, is responsible for the differential effect.

Heating the style 25.5/18°C extract up to 70°C, increases the length of the pollen tubes compared to the untreated 25.5/18°C extract. The same is true for the style 19.5/18°C extract if heated up to 100°C (*table* 2).

Application of protease K to the style 25.5/18°C extract decreases the length of the pollen tubes growing in that extract and there is no longer any difference

in pollen tube length between the enzyme treated 25 5 18 C extract and the untreated 19 5 18 C extract (*fig 4* and *table 2*) No change in pollen tube length could be observed when the style extracts were treated with RNA'se or DNA'se (*table 2*)

4 DISCUSSION

The low temperature regime (19 5 18 C) during style development and progamic phase does not only prolong the recognition process in *Petunia* but also alters it in such a way that acceptance of the compatible pollen tubes (tubes resulting after a cross-pollination) does not occur (VAN HERPEN & LINSKENS 1981) According to the model of VAN DER DONK (1975), the pollen genome is not activated because the style specific polypeptides are probably not synthesized If this "incompatible-like" situation exists it can be used to determine, with the help of the excised-style technique, whether or not proteins, synthesized in the style before pollination, could change that "incompatible-like" situation into a compatible one Our experiments (*table 2*) prove the existence of S_1S_1 stylar proteins, only synthesized in the style before pollination under a temperature regime of 25 5 18 C, which have an effect on the length of the S_1S_2 cross-pollen tubes in semi-vitro culture So the "incompatible-like" situation, after a compatible pollination, as first postulated by VAN HERPEN & LINSKENS (1981) is confirmed However, it is not impossible that stylar polypeptides are formed during pollination, but according to the experiments of VAN HERPEN (1981), the gene activity and subsequent polypeptide synthesis before the progamic phase is independent of the kind of pollination The translation of masked messenger RNA to stylar polypeptides found by VAN DER DONK (1975) during the progamic phase is completely evaded

The conclusion of SHARMA & SHIVANNA (1982) that "unpollinated pistil contains self-incompatibility factors which affect *in vitro* germination and tube growth of self- and cross-pollen differently" gives more than reasonably can be concluded from those few experiments performed, and so the remark on the stylar polypeptides of VAN DER DONK (1975) is rather preliminary

The use of DNA'se and RNA'se in combination with the unpollinated T_5U extracts had no effect on the length of the W166K pollen tubes It is possible, unless the excised style technique is invalid, that the pollen genome, activated by the stylar polypeptides, does not activate the Stylar S-gene but results in a more and better use of the compounds available for the pollen tubes in the unpollinated style extracts This would be in contrast with the findings of VAN DER DONK (1975) who postulated the onset of the stylar S-gene by the activated pollen genome

The proteins in the style extract of styles developed at 25 5/18 C promote more or inhibit less than the proteins in the 19 5/18 C style extract The experiments with protease K indicate that the 19 5/18 C proteins have no effect, and because of that the 25 5 18 C proteins must have a promoting effect on the

length of the protruding pollen tubes However, it is still possible that there are inhibitory proteins in the 25 5 18 C extract Heating up the 25 5/18 C extract to 70 C has an effect on the pollen tube length, so it is not unlikely that an inhibiting compound in the style extract is inactivated

The length of the pollen tubes resulting from a compatible pollination is certainly not determined by the amount of available free carbohydrates, because no differences could be detected between pollen tubes growing in the free carbohydrate extract of unpollinated styles developed either at the low or high temperature regime (*table 1*) Our excised style technique gives a completion on the previously made assumption that free carbohydrate content in the style could not be correlated with the length of the cross-pollen tube (VAN HERPEN, 1983) Determination of the free carbohydrate content in styles developed at the low temperature regime and supplemented with 24 hours of the high temperature regime (25 5/18°C) revealed a sudden drop in the free carbohydrate content of the style in the first day after the high temperature treatment, immediately followed by a rise in free carbohydrate content in the next two days, and again in a drop and subsequent rise in free carbohydrate content (VAN HERPEN, in preparation) These results could also not be correlated with the greater length of the pollen tubes growing in those styles as described previously (VAN HERPEN & LINSKENS 1981)

Our excised style technique is a good and reliable method for studying pollen-style interactions through pollen tube growth

REFERENCES

BREWBAKER, J L & S K MAJUMDER (1961) Cultural studies of the pollen population effect and the self-incompatibility inhibition *Amer J Bot* **48** 457–464

— & B H KWACK (1963) The essential role of calcium ion in pollen germination and tube growth *Amer J Bot* **50** 859–865

DONK, J A W VAN DER (1975) *Molecular biological aspects of the incompatibility reaction in Petunia* Thesis, University Nijmegen

DUBOIS, M , K A GILLES, J K HAMILTON, P A REBERS & F SMITH (1956) Colorimetric method for determination of sugars and related substances *Analyt Chem* **28** 350–356

HERPEN, M M A VAN (1981) Effect of season, age and temperature on the protein pattern of pollen and styles in Petunia hybrida *Acta Bot Neerl* **30** 277–287

— (1983) Temperature, Petunia stylar ultrastructure and free carbohydrates *Proceedings 7th International Symposium on Fertilization and Embryogenesis in Ovulated Plants* pp 227–230 14–17th June 1982 Račkova Dolina, Czechoslovakia

— (in preparation) The effect of temperature on the ultrastructure of transmitting tissue in the style and on the free carbohydrate content in pollen and styles of Petunia hybrida

— & H F LINSKENS (1981) Effect of season, plant age and temperature during plant growth on compatible and incompatible pollen tube growth in Petunia hybrida *Acta Bot Neerl* **30** 209–218

Kunitz, M (1940) Crystalline Ribonuclease *J Gen Physiol* **24** 15–32

LINSKENS, H F (1955) Physiologische Untersuchungen der Pollenschlauch-Hemmung selbststeriler Petunien *Z Bot* **43** 1–44

— & K ESSER (1959) Stoffaufnahme der Pollenschlauche aus dem Leitgewebe des Griffels *Proc Kon Ned Akad Wetens (Amsterdam)* **C62** 150–154

LOWRY, O. H., N J ROSEBROUGH, A L. FARR & R. J. RANDALL (1951). Protein measurement with the Folin phenol reagent *J. Biol. Chem* **193**. 265–275.

NIIMI, Y (1982) Studies on the self-incompatibility of Petunia hybrida in excised-style culture An attempt at improving a technique in excised-style culture *Euphytica* **31** 787–793.

PLUIJM, J. VAN DER & H. F LINSKENS (1966) Feinstruktur der Pollenschlauche im Griffel von Petunia. *Zuchter* **36**. 220 224

SHARMA, N & K R SHIVANNA (1982): Effect of pistil extracts on in vitro responses of compatible & incompatible pollen in Petunia hybrida Vilm. *Indian J exp Biol* **20**. 255–256

SCHOCH-BODMER, H (1932) Methoden zur Ermittlung der Wachstumsgeschwindigkeit der Pollenschläuche im Griffel *Verh Schweiz. Naturforsch Ges.* pp 368–370

STRAUB, J (1946). Zur Entwicklungsphysiologie der Selbststerilitat von Petunia. *Z. Naturforsch.* **1** 287–291.

HEAT PRODUCTION OF POLLINATED AND UNPOLLINATED PISTILS OF PETUNIA HYBRIDA

SUMMARY

Immediately after pollination, the contribution of T_2U (S_3S_3) or *W166K* (S_1S_1) pollen to the total heat production of the pollinated T_2U pistils is 1.5% and this percentage decreases to zero in approximately one hour. One hour after insertion of the ampoules the total amount of heat produced is the same for pollinated and unpollinated pistils.

From one hour on after pollination, compatible and incompatible pollinated pistils have an increase in heat production as compared to unpollinated pistils of the same clone. This increase is greater in incompatible than in compatible pollinated pistils.

T_2U pistils pollinated with either viable or non-viable T_2U pollen have the same pattern of heat production, in contrast with T_2U pistils pollinated with viable or non-viable *W166K* pollen. T_2U pistils pollinated with non-viable *W166K* pollen act, as far as the production of heat is concerned, as unpollinated pistils.

1. INTRODUCTION

It was observed that the protein and RNA metabolism in the ovaries of *Petunia* were different after cross- and self-pollination (LINSKENS 1973, DEURENBERG 1977). This distinction was induced long before the pollen tubes had reached the ovary. DEURENBERG (1977) concluded from these observations that recognition signals, probably of an electrical nature, had to be emitted from the stigma or the style in order to evoke the differential metabolic processes.

The kind of pollination proved to have an effect on the generation of different bioelectric-potential-changes in the style (LINSKENS & SPANJERS 1973, SPANJERS 1981) which could have been responsible for the metabolic changes in the ovary as observed (DEURENBERG 1977).

When we accept that bioelectric signals in the style induce metabolic changes and not only in the ovary, it has to be possible to obtain more information about the recognition reaction as part of the pollen-pistil interaction via differential metabolic processes induced by different kinds of pollen.

Some indications for the validity of this idea are in the observation that self- and cross-pollinated styles have a different oxygen uptake, which is already distinct two hours after pollination (LINSKENS 1955).

Instead of using the Warburg we worked with the Bio-Activity monitor of LKB, which reduced the time necessary for equilibration significantly, and that was important because the recognition reaction had to be studied in the first hours after pollination (LINSKENS 1955, 1975).

This Bio-activity monitor, a calorimeter (PRAT 1962) measures heat production instead of gas exchange but heat can be as good an expression of metabolic activity as O_2 and CO_2.

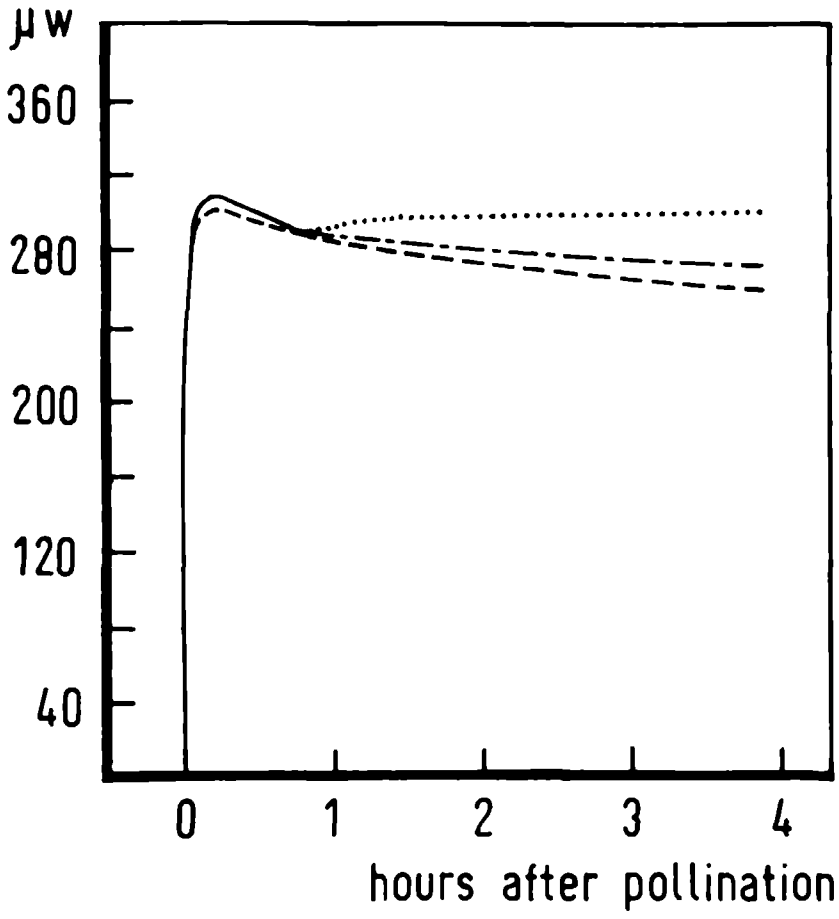


Fig 1 Time course of heat production of five pollinated or not-pollinated pistils plus 5 mm receptacle T_2U styles pollinated with viable or non-viable T_2U pollen (self-incompatible pollination) — · — · T_2U styles pollinated with viable *W166K* pollen (cross-compatible pollination) — — — — Unpollinated T_2U styles or T_2U styles pollinated with non-viable *W166K* pollen

2 MATERIALS AND METHODS

Pistils were collected from T_2U (S_1S_3) summer cuttings grown, from the moment of cutting in a plant growth chamber with day/night regime of 19 5 /8 C (VAN HERPEN & LINSKENS 1981)

T_2U (S_2S_3) and *W166K* (S_1S_1) pollen were from plants developed at the high temperature regime of 25 5 /8 C

Non-viable pollen, that forms no pollen tubes after *in vivo* or *in vitro* pollinations, was pollen kept at room temperature for more than one year Viable pollen on the other hand was treated as described previously (VAN HERPEN & LINSKENS 1981, VAN HERPEN 1981)

Heat exchange was measured with the LKB 2277 Bio-Activity monitor, calibrated at 25 0 C

Five pistils plus 5 mm receptacle with or without 0 3 mg pollen on the stigma were put into 5 ml steel ampoules filled with 500 µl milli-q, which were pre-equilibrated at 25 0 C in a stove

Two ampoules, one with and the other without pistils, were lowered into the Bio-Activity monitor without further equilibration and measured against each other

3 RESULTS (see *fig 1*)

Pollinated styles have a higher heat production than unpollinated or cross-pollinated pistils even if non-viable pollen is used

Cross-pollinated pistils have a higher heat production than unpollinated and a lower heat production than self-pollinated styles when viable pollen is used Non-viable pollen has, in contrast with viable pollen, no effect on the heat exchange of the compatible pollinated T_2U pistils and the heat production of those pistils equals that of unpollinated pistils

The heat production of T_2U and *W166K* pollen is the same, so the contribution of the pollen to the total heat exchange of the pollinated style is not different for self or cross-pollinated pistils when equal amounts of pollen are used

4 DISCUSSION

The observation by LINSKENS (1975) that recognition takes place 0-3 hours after the primary contact of the pollen with the stigma seems to be confirmed by our experiments in which we observe a fast recognition of the pollen, expressed in a higher heat production of the pollinated pistils as compared to the unpollinated ones (*fig 1*)

The heat production after self-pollination, as can be concluded from the experiment with non-viable pollen, has its origin in the pistil We have the impression that an incompatible pollination puts up the pistil

either to spill much energy or to build a barrier against the possibly coming pollen tubes.

After cross-pollination the heat exchange either has to depend on the higher activity of the pollen tubes or on the interaction of pollen and pistil, because non-viable pollen does not and viable pollen does have an effect on the heat exchange.

So we have the strong impression that self- and cross-pollinated pistils differ in the measure of interaction between the pollen(tube) and the pistil: more interaction after cross-pollination than after self-pollination. This difference in interaction seems to be confirmed by the observations of VAN HERPEN & LINSKENS (1981) that temperature treatment of the pistil before the start of the progamic phase does have an effect on the length of the pollen tubes resulting from a compatible pollination and has no effect on the length of the tubes resulting from an incompatible pollination.

When this hypothesis of differential interaction is true pollen tube length after an incompatible pollination does depend strongly on the condition of the pollen itself because stylar energy or resources are not converted completely to the growing pollen tube but are used in great extent for other processes resulting in a high heat production.

After a compatible pollination with viable pollen on the other hand there seems to be more interaction than after an incompatible pollination, and whether it is this interactive process that consumes less energy in comparison with the incompatible pollination or the fact that the moderate acceleration of the heat production leaves more energy for the interaction of the pistil with the pollen or pollen tube is not clear. More interaction means that the tube length of pollen resulting from a compatible pollination does not completely depend on the condition of the pollen but also on the condition of the style (VAN HERPEN & LINSKENS 1981).

SPANJERS (1981) observed in experiments with *Lilium* that killed self-incompatible pollen, in contrast with killed cross-compatible pollen, prevents the generation of any bioelectric response and he subsequently proposes that the pollen is not recognized at all or is no longer able to excitate a response in the style. In *Petunia* on the other hand, only the non-viable cross-compatible pollen in contrast with the non-viable self-incompatible pollen is not able to excitate a response in the style and that is totally opposite from the observations in *Lilium*. It is possible that the bioelectric response SPANJERS (1981) observed in *Lilium* is present in *Petunia* but does not induce the metabolic changes we observed or that non-viable pollen is not identical with killed pollen or that the two plant species can not be compared.

Much work has to be done about the recognition signals in the first hours after pollination and the metabolic processes such as heat exchange which seems to be a good tool for studying pollen-style interactions.

REFERENCES

DEURENBERG, J J M (1977) *Aktivering van het vruchtbeginsel van Petunia* Thesis, University Nijmegen

HERPEN, M M A VAN (1981) Effect of season, age and temperature on the protein pattern of pollen and styles in Petunia hybrida *Acta Bot Neerl* **30** 277-287

— & H F LINSKENS (1981) Effect of season, plant age and temperature during plant growth on compatible and incompatible pollen tube growth in Petunia hybrida *Acta Bot Neerl* **30** 209-218

LINSKENS, H F (1955) Physiologische Untersuchungen der Pollenschlauch-Hemmung selbststeriler Petunien *Z Bot* **42** 1-44

— (1973) Activation of the ovary *Caryologia* Vol **25**, Suppl , pp 27-41

— (1975) Incompatibility in Petunia *Proc R Soc Lond* **B188** 299-311

— & A W SPANJERS (1973) Changes of the electrical potential in the transmitting tissue of Petunia-styles after cross- and self-pollination *Incomp Newsletter* **3** 81-85

PRAT, H (1962) Methodes calorimetriques pour l'analyse des vegetaux In *Moderne Methoden der Pflanzen Analyse* Pp 359-367 (Eds H F LINSKENS & M V TRACEY) Springer-Verlag, Berlin-Gottingen-Heidelberg

SPANJERS, A W (1981) Bio-electric potential changes in the style of Lilium longiflorum Thunb after self- and cross-pollination of the stigma *Planta* **153** 1-5

SUMMARY

Environmental conditions during the growth of *Petunia hybrida* have an influence on the interactive processes between pollen and style during the progamic phase. The time of the year in which the plant cuttings are prepared, the temperature during plant growth and the age of the plant have an effect on the condition of the plant and because of that on its style and pollen.

The length of the pollen tubes after 24 hours growth on the plant depends on the temperature during the progamic phase and on the condition of the pollen and style which on its turn is determined by environmental conditions before pollination (VAN HERPEN & LINSKENS 1981). The kind of pollination (incompatible or compatible) however, determines to which extent the condition of the pollen and the style contributes to the ultimate length the pollen tubes can reach in 24 hours growth on the plant. After an incompatible pollination only the condition of the pollen has an effect on the ultimate pollen tube length;after a compatible pollination however, it is the condition of the style. Pollen developed at the day/night temperature regime of 25.5/18°C make a longer pollen tube than pollen developed at the lower temperature regime of 19.5/18°C, when those differently treated pollen are tested in an incompatible pollination. On the other hand in a compatible pollination the longest pollen tubes can be found in those styles developed at the highest temperature regime of 25.5/18°C. Even a 24 h temperature enhancement from 19.5/18°C to 25.5/18°C given to the style before the start of the progamic phase is so strong that the impact is only then gone when the time lapse between enhancement and pollination exceeds 4 weeks.

Because of the fact that the kind of pollination determines whether the temperature before the progamic phase expresses itself via the pollen or the style in the ultimate length of the pollen tubes, made it ideal to study the compatible and incompatible pollination with temperature as the main variable (VAN HERPEN 1981, 1983, 1984a,b).

The qualitative protein composition of pollen and style extracts, determined with immunoelectrophoretic techniques, depends on all the environmental factors mentioned above:season, temperature during plant growth and age of the plant. The total amount of protein in pollen was higher when the pollen developed at the 25.5/18°C regime instead of the lower temperature regime of 19.5/18°C; styles developed at different temperature regimes only have the above mentioned qualitative difference in protein composition (VAN HERPEN 1981).

The effect of the high temperature regime on the amount of available fats, free carbohydrates and phytic acid in pollen is identical with that of the low temperature regime when the total amount of lipids is concerned, pollen developed at the 25.5/18°C regime however has more free carbohydrates and phytic acid than pollen developed at the 19.5/18°C temperature regime (VAN HERPEN 1984b). So a plausible conclusion was

that pollen developed at the 25 5/18 C temperature regime has more reserve material and because of that makes longer pollen tubes than pollen developed at the lower temperature regime

The correlation between temperature during style development and the amount of lipids, free carbohydrates and intercellular substance was also investigated styles developed at the low regime posess more lipids and free carbohydrates but less intercellular substance than styles developed at the high temperature regime (VAN HERPEN 1983, 1984b)

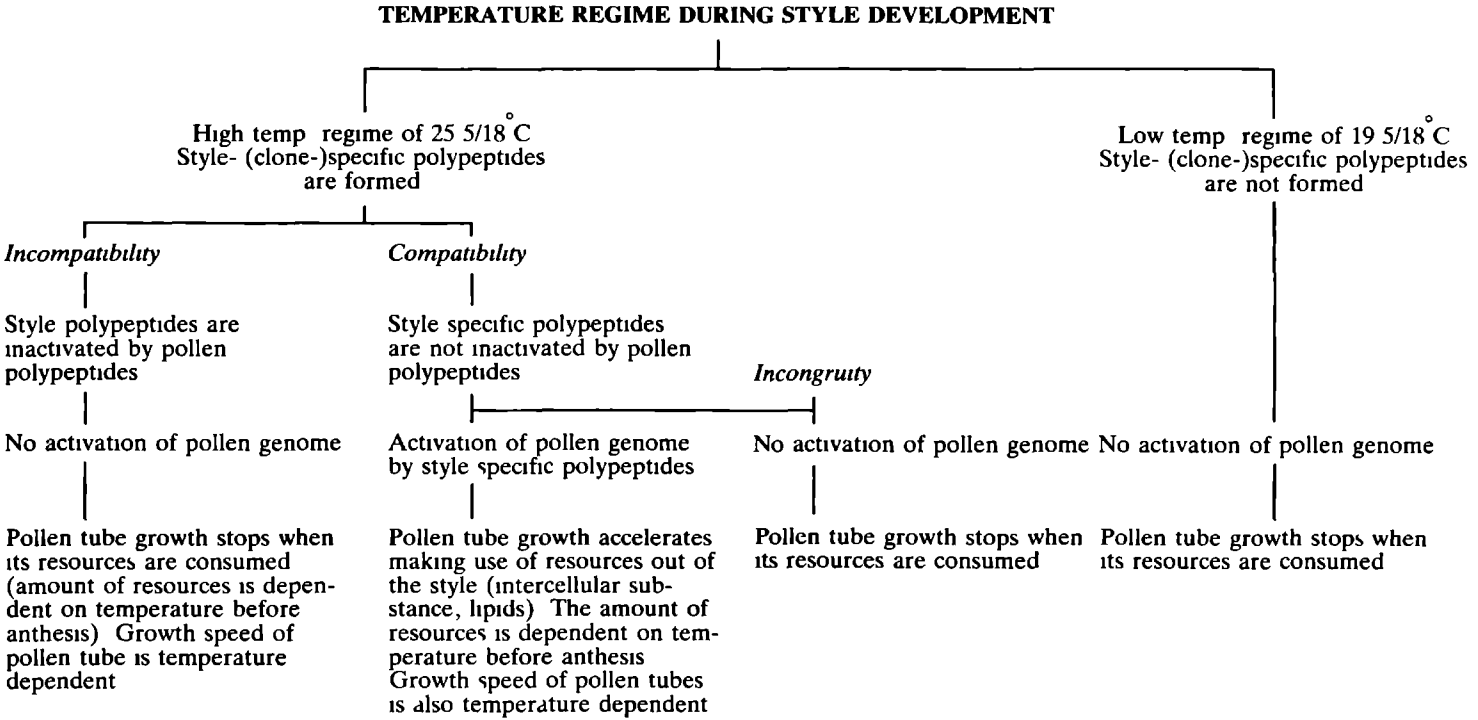
The following question was which of the above mentioned changes in style compounds is responsible for the difference in length of the pollen tubes growing through those styles An experiment was set up in which the effect of the substances out of those styles could be tested on growing pollen tubes (VAN HERPEN 1984a) The final result was that the pollen tube length could be regulated by proteins which presence in the style is directly dependent on the temperature during style development

The model of VAN DER DONK (1975), which was till that moment the most fitting model for our results, could be changed in order to make it more compatible with the newly found data

-Incompatible pollination after an incompatible pollination the growth speed of the pollen tubes is temperature dependent and the maximum length of the pollen tubes after 24 hours growth on the plant (style) is determined by the amount of available reserves in the pollen itself, and this amount depends on the temperature during pollen development Pollen tubes resulting from an incompatible pollination can not make use of the reserves out of the style because the pollen genome is not activated by the style specific polypeptides These style specific polypeptides are in the case of an incompatible pollination, inactivated by the pollen specific polypeptides

-Compatible pollination after a compatible pollination the style specific polypeptides are not inactivated by the pollen specific polypeptides and activate the pollen genome, so the pollen tubes are able now to mobilize the reserve material out of the style and make longer pollen tubes The amount of reserve material in the style as well as the synthesis of style specific polypeptides depend on the temperature before and during pollination When the temperature during style development is low no style specific polypeptides can be synthesized and an incompatible like situation is the result no activation of the pollen genome which results in a limited pollen tube length

In contrast with the original model of VAN DER DONK (1975) this new model is based on the fact that the style specific polypeptides can be present before pollination


TEMPERATURE REGIME DURING STYLE DEVELOPMENT
High temp regime of 25 5/18 °C
Style- (clone-)specific polypeptides are formed
Low temp regime of 19 5/18 °C
Style- (clone-)specific polypeptides are not formed
Incompatibility
Compatibility
Style polypeptides are inactivated by pollen polypeptides
Style specific polypeptides are not inactivated by pollen polypeptides
Incongruity
No activation of pollen genome
Activation of pollen genome by style specific polypeptides
No activation of pollen genome
No activation of pollen genome
Pollen tube growth stops when its resources are consumed (amount of resources is dependent on temperature before anthesis) Growth speed of pollen tube is temperature dependent
Pollen tube growth accelerates making use of resources out of the style (intercellular substance, lipids) The amount of resources is dependent on temperature before anthesis Growth speed of pollen tubes is also temperature dependent
Pollen tube growth stops when its resources are consumed
Pollen tube growth stops when its resources are consumed

SAMENVATTING

Uitwendige omstandigheden tijdens de groei van *Petunia hybrida* blijken een grote invloed te hebben op de processen tijdens de interactie van pollen en stijl. De tijd van het jaar waarin de stek gemaakt wordt, de temperatuur tijdens de groei alsmede de leeftijd van de plant hebben ieder voor zich een invloed op de conditie van de plant en als zodanig invloed op de stijl en het pollen. De conditie nu van pollen en stijl, bepaald door factoren vóór de bestuiving, is tezamen met de temperatuur tijdens de bestuiving bepalend voor de lengte van de pollenbuizen (VAN HERPEN & LINSKENS 1981).

De mate waarin echter de stijl en het pollen invloed kunnen uitoefenen op de lengte van de pollenbuizen hangt af van het feit of het een incompatibele- of zelf-bestuiving dan wel een compatibele- of kruis-bestuiving betreft. Als we bijvoorbeeld kijken naar het effect van de temperatuur tijdens de ontwikkeling van stijl en pollen op de uiteindelijke pollenbuis-lengte dan blijkt dit, voor wat betreft de incompatibele en compatibele bestuiving, terug te voeren op de veranderde conditie van respectievelijk pollen en stijl. Pollen ontwikkeld bij een temperatuur regiem van 25,5/*18*°*C* in een dag/nacht ritme van 18/6 uur vormt een langere buis dan pollen ontwikkeld bij het lagere temperatuur regiem van 19,5/*18*°*C* mits dit pollen gebruikt wordt voor zelf-bestuiving.

Een hogere temperatuur tijdens de ontwikkeling van de stijl heeft alleen in het geval van een compatibele bestuiving effect op de lengte van de pollenbuis. De invloed van een eenmalige hoge temperatuur (25,5/*18*°*C*) vóór de bestuiving op de stijl en daarmee op de pollenbuis-lengte is bij een compatibele bestuiving dermate sterk dat het effect van deze temperatuur-verhoging op de lengte van de pollenbuizen dan pas niet meer merkbaar is als de periode tussen de tijdelijke temperatuur-verhoging en de dag van bestuiving meer dan vier weken bedraagt.

Daar de aard van de bestuiving bepaalt of de temperatuur vóór de bestuiving zich via het pollen dan wel via de stijl manifesteert in de lengte van de pollenbuis, is voor ons aanleiding geweest ons onderzoek meer en meer toe te spitsen op deze ene variabele om zodoende meer inzicht te krijgen in de compatibele en incompatibele bestuiving (VAN HERPEN 1981, 1983, 1984a,b).

De kwalitatieve eiwitsamenstelling van pollen en stijl, bepaald met behulp van immunoelectrophoretische technieken, blijkt in sterke mate afhankelijk van bovengenoemde factoren als leeftijd van de plant, temperatuur tijdens de groei en seizoen. Een van de eerste gegevens van het onderzoek was dat de totale hoeveelheid eiwitten in het pollen hoger is indien dit pollen zich ontwikkelt bij de hoge temperatuur van 25,5/*18*°*C*; stijlen ontwikkeld bij de twee verschillende temperatuur regiems vertonen buiten het al reeds vermelde kwalitatieve verschil echter geen verschil in kwantitatieve eiwitsamenstelling (VAN HERPEN 1981).

Voor wat betreft het pollen is er gekeken naar het effect van het hoge temperatuur-regiem van 25,5/18°C op de hoeveelheid aanwezige vetten, vrije koolhydraten en fytinezuur in vergelijking met het lage temperatuur regiem van 19,5/18°C: er is geen verschil tussen beide regiems voor zover het de kwantitatieve lipidensamenstelling betreft, pollen ontwikkeld bij het hogere temperatuur regiem bevat wel meer vrije koolhydraten en fytinezuur dan pollen ontwikkeld bij het lagere temperatuur regiem (VAN HERPEN 1984b). Samenvattend kan gezegd worden dat pollen ontwikkeld bij het temperatuur regiem van 25,5/18°C meer reservestoffen heeft en daardoor waarschijnlijk langere pollenbuizen vormt dan pollen ontwikkeld bij het lagere regiem van 19,5/18°C.

Voor wat betreft de stijl is er gezocht naar de correlatie tussen de temperatuur en de hoeveelheid vetten, vrije koolhydraten en intercellulair materiaal (VAN HERPEN 1983, 1984b), de keuze van deze laatste factor hangt samen met het feit dat de pollenbuis door dit materiaal heen moet groeien. Stijlen ontwikkeld bij de lage temperatuur van 19,5/18°C bezitten meer lipiden en vrije koolhydraten doch minder intercellulaire substantie in vergelijking met stijlen ontwikkeld bij de hoge temperatuur van 25,5/18°C.

Om nu te bepalen welke van bovengenoemde veranderingen in samenstelling van de stijl verantwoordelijk was voor het verschil in pollenbuislengte van de door deze stijlen heengroeiende pollenbuizen is gebruik gemaakt van een opstelling waarin het directe effect van deze stoffen op de groeiende pollenbuizen in *semi-vitro* cultuur getest kon worden (VAN HERPEN 1984a). Bepalend voor een grotere lengte van de pollenbuizen zijn eiwitten, waarvan het al of niet aanwezig zijn in de stijl gecorreleerd is met de temperatuur tijdens de ontwikkeling van deze stijl.

Het door VAN DER DONK (1975) in zijn dissertatie opgestelde model bleek, aangepast aan onze gevonden resultaten, van alle bestaande modellen toch het meest hanteerbare te zijn.

-Incompatibele bestuiving: na een incompatibele bestuiving is de groeisnelheid van de pollenbuizen afhankelijk van de temperatuur en wordt de maximale lengte van de buizen bepaald door de beschikbare hoeveelheid reservestoffen in het pollen; de uiteindelijke hoeveelheid reservestoffen hangt af van de temperatuur tijdens de pollen ontwikkeling. De groeiende pollenbuizen kunnen geen gebruik maken van de reservestoffen in de stijl omdat het pollen-genoom niet aangeschakeld wordt door de in de stijl voorkomende kloon-specifieke eiwitten. In het geval van een incompatibele bestuiving zijn deze stijl-specifieke eiwitten namelijk weggevangen door pollen-specifieke eiwitten.

-Compatibele bestuiving: na een compatibele bestuiving worden de in de stijl aanwezige stijl- of kloon-specifieke eiwitten (polypeptiden) niet geïnactiveerd door de in het pollen voorkomende pollen-specifieke eiwitten. Deze niet-geïnactiveerde stijl-eiwitten activeren het pollen-genoom met als gevolg dat de pollenbuizen nu wel in staat zijn om de reservestoffen uit de stijl te mobiliseren waardoor de lengte van de

pollenbuizen positief beïnvloed wordt in vergelijking met de incompatibele situatie.

De vorming van de reservestoffen in de stijl zowel als van de stijl specifieke eiwitten wordt bepaald door de temperatuur voor en na de bestuiving. Als nu de temperatuur tijdens de ontwikkeling en bestuiving van de stijl laag is (19,5/18°C in plaats van 25,5/18°C) dan worden er geen stijl-specifieke eiwitten gevormd en ontstaat er een situatie die op een incompatibele situatie lijkt: er treedt ook nu geen activering van het pollen-genoom op, met als gevolg een beperkte pollenbuislengte.

In tegenstelling tot het model van VAN DER DONK (1975) is dit model gebaseerd op het al voor de bestuiving aanwezig zijn van de stijl-specifieke polypeptiden.

REFERENCES/LITERATUUR

DONK, J.A.W.M.VAN DER (1975). *Molecular biological aspects of the incompatibility reaction in Petunia.* Thesis, University Nijmegen.

HERPEN, M.M.A.VAN (1981): Effect of season, age and temperature on the protein pattern of pollen and styles in Petunia hybrida. *Acta Bot. Neerl.* **30**:277-287.

— & H.F.LINSKENS (1981): Effect of season, plant age and temperature during plant growth on compatible and incompatible pollen tube growth in Petunia hybrida. *Acta Bot. Neerl.* **30**:209-218.

— (1983): Temperature, Petunia stylar ultrastructure and free carbohydrates. *Proceedings 7th International Symposium on Fertilization and Embryogenesis in Ovulated Plants.* 14-17th June 1982. Račkovà Dolina (Czechoslovakia). Pp.227-230.

— (1984a): Extracts from styles, developed at different temperatures, and their effect on compatibility of Petunia hybrida in excised-style culture. *Acta Bot. Neerl.* **33**:195-203.

— (1984b). *Environment and pollen-style interaction.* Thesis, University Nijmegen.

CURRICULUM VITAE

Marinus M A van Herpen werd geboren op 8 augustus 1949 te 's-Hertogenbosch Na de lagere school genoot hij voortgezet onderwijs aan het eveneens te 's-Hertogenbosch gevestigde St Janslyceum In 1968 behaalde hij het diploma HBSb en startte in hetzelfde jaar met de studie biologie en natuurkunde (B3) aan de Katholieke Universiteit van Nijmegen Eind 1971 werd het kandidaatsexamen (B3) afgelegd, met de aantekening "met genoegen" Als doctoraalprogramma koos hij twee hoofdvakken en een bijvak respectievelijk biofysica en plantenfysiologie alsmede plantenoecologie Binnen de biofysica werd onder leiding van Prof dr E Eijkman zintuig- en gedrags-fysiologisch onderzoek verricht aan de olifantsvis Gnatonemus Petersii Het plantenfysiologisch hoofdvak onder leiding van Prof dr ir J F G M Wintermans was gericht op de spill-over van energie tussen lichtsysteem I en II In het vakgebied van de plantenoecologie werd onder leiding van Prof dr H F Linskens de inbreng van de plantenoecologie in de criminologie bestudeerd Gedurende de studiejaren 1973 en 1974 was hij zowel assistent bij het practicum plantenfysiologie op universitair niveau als bij een trainingscursus voor leraren biologie op middelbaar niveau Begin 1974 werd het doctoraalexamen met pedagogische aantekening behaald Van maart 1974 tot september 1975 vervulde hij zijn militaire dienstplicht als onderofficier civiele vakopleiding in de rang van sergeant In de periode 1974-1984 was hij werkzaam als leraar biologie aan middelbare scholen in Schijndel, Nijmegen en Apeldoorn en als docent voor de vakgebieden biologie en natuurkunde aan de Pedagogische Akademie in Schijndel en de Hogere en Middelbare Laboratorium School in Oss Per 1 november 1977 werd hij benoemd tot wetenschappelijk medewerker in deeltijd betrekking aan het Botanisch Laboratorium I van de K U Nijmegen welke betrekking hij tot op heden vervult Naast de onderzoeks-opdracht waaruit het voorliggende proefschrift resulteert omvatte deze betrekking ook onderwijs-taken In de reeks van jaren participeerde hij in de cursussen fotosynthese, bio-monitoring van milieu verontreinigingen, cryptogamen, fotosynthese-research cursus, PAO cursus fotosynthese, fytopathologie en plantenfysiologie In bovengenoemde periode volgde hij de cursussen biochemische scheidings-technieken, tekstverwerking en pascal Sinds februari 1984 is hij naast zijn part-time baan in Nijmegen in eigen vrije tijd werkzaam aan de stichting ITAL te Wageningen in een plantecel-biotechnologisch project Per 1 september 1984 is hij tevens werkzaam als studiebegeleider aan de Open Universiteit Josephine A M Middeldorp is sinds 1978 zijn partner

STELLINGEN

I

Door het ontbreken van gegevens betreffende de uitwendige omstandigheden tijdens de groei van proefplanten zijn de resultaten van verschillende onderzoekers op het gebied van de biochemie van de incompatibiliteit onvergelijkbaar

Dit proefschrift

II

Nieuwe geavanceerde apparatuur leidt vaak tot een schat aan nieuwe gegevens c q vele publicaties Als nu het aantal publicaties een maat wordt voor de hoeveelheid toe te wijzen geldmiddelen, dan is de kans erg groot dat investeren in hoog gekwalificeerde onderzoekers niet langer het grootste profijt zal opleveren

III

Gezien de beperkte hoeveelheid tijd beschikbaar voor het bewerken van een proefschrift zal een promovendus in de verleiding komen vaak beproefde standaardbepalingen uit de literatuur te prefereren boven minder beproefde maar meer voor zijn doel geeigende methodieken

IV

De met betrekking tot de citroenzuurcyclus opgestelde 'flow-chart' van ROBERTS (1982) kan door de sterke vereenvoudiging verkeerd geinterpreteerd worden

ROBERTS, M B V (1982) *Biology a functional approach* 3rd edn p 107 Nelson, London

V

In het onderzoek naar de invloed van eiwitten op de lengte van de pollenbuis, trekken SHARMA & SHIVANNA hun conclusies op grond van de veronderstelling dat zij te maken hebben met drie vergelijkingen met drie onbekenden Dit is onjuist daar het hier een combinatie van twee vergelijkingen met drie onbekenden betreft

SHARMA, N & K R SHIVANNA (1982) *Indian J Exp Biol* **20** 255-256

VI

Extrapolatie van resultaten uit vergelings- of verouderingsexperimenten aan bladstukjes naar het intacte uitgangsmateriaal is vaak niet geoorloofd

VII

Gezien de veranderende leeftijdsopbouw van de Nederlandse bevolking is het niet langer vanzelfsprekend de aan aardgas toe te voegen concentratie kunstmatige geurstoffen vast te stellen op grond van gegevens verkregen met studenten als proefpersonen

KOSTER, E P (1978) Geuren en stank *Intermediair* **10** 25-29

VIII

Zowel BUSER & MATILE als DURR *et al* isoleren vacuolen middels lyse van protoplasten met behulp van DEAE-dextran De gebruikte DEAE-dextran concentraties verschillen echter een factor 200 Het verschil in uitgangsmateriaal is niet de enige hiervoor verantwoordelijke parameter

DURR, M , T BOILER & A WIEMKEN (1975) *Arch Microbiol* **105** 319-327
BUSER, CH & PH MATILE (1977) *Z Pflanzenphysiol* **82** 462-466

IX

In het biologie onderwijs geven de begrippen zelf- en kruisbestuiving aanleiding tot verwarring

X

Brievenbocken zijn duidelijk uit de mode waar het privé-correspondentie betreft In tegenstelling daarmee worden zakelijke en wetenschappelijke mededelingen steeds stereotieper geformuleerd Tekstverwerkende systemen zijn daarom niet zo modern als wel gedacht wordt

XI

Verschil in maatschappelijke status moet er zijn!
Het verschil echter tussen de ene handwerker (gr cheir-ourgos) en de andere is soms wel wat erg groot

M M A VAN HERPEN, Nijmegen 13 december 1984