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Review

The role of peroxidases in pistil-pollen interactions

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Contents

Summary. The majority of pistil peroxidases are involved in processes related to growth, development and senescence. Only the tissue specific peroxidases in the transmitting tissue of the style may play a direct role in the regulation of pollen tube growth. The pollen peroxidases may function mainly in growth regulation and tube wall formation and play a role in the interaction between pollen and pistil by metabolizing the phenolic compounds in the pistil.

Key words: Peroxidase activity – Peroxidase isoenzymes – Stigma – Style – Pistil

1 Introduction

The fertilization process in Angiosperms can be divided into different phases: pollen germination, penetration of the pollen tubes into the stigma, growth of the pollen tubes in the style, and syngamy (Linskens 1968; Linskens and Kroh 1970).

The interaction between the pistil and pollen during the fertilization process includes several physiological events, such as wound reactions, nutrition of pollen tubes, incompatibility reaction, activation and growth of the ovary, translocation phenomena, accelerated senescence, wilting and abscission. All these reactions are accompanied by phase specific metabolic alterations including the changes in enzyme activities (Roggen 1967; Linskens et al. 1969). However, most of the studies on enzyme activities have been merely descriptive and provide no definite conclusions, for instance how the changes in enzyme activities are induced, and to what extent such changes contribute to the physiological processes which accompany the pistil-pollen interaction.

Only in the case of peroxidases have attempts been made to solve some of these problems. The peroxidase is suitable as a model enzyme for studying the pistil-pollen interaction because of the following reasons:
– The peroxidase isoenzyme composition of *Nicotiana alata* styles was assumed to be related to the S (self-incompatible) genotype (Pandey 1967).
– It has been proposed that the peroxidase is physiologically active in the interaction between the pathogenic

organisms and host plants (see review Gaspar et al. 1982). This interaction exhibits several analogies with those occurring between the pistil and pollen (Linskens 1968 b; Hogenboom 1973).

– Peroxidase is thought to perform a wide variety of catalytic functions (Gaspar et al. 1982) because of its high number of isoenzyme forms that differ in hydrogen donor specificity and localization (Darimont and Baxter 1973; Marklund et al. 1974; Mäder et al. 1980).

A number of these functions of peroxidase may play a role in the interaction between pistil and pollen. One can think, in this connection, of inactivation of IAA (Ray 1958), destruction of toxic hydrogen peroxide (Cohen and Hochstein 1963) and formation of this substance (Mäder et al. 1980), ethylene formation (Mapson and Wardale 1973), hydroxylation of proline in cell walls (Ridge and Osborne 1970), lignification (Vance et al. 1976), ion translocation (De Jong 1966; Niedermeyer 1975) and degradation of flavonols (Hösel et al. 1975).

But first of all, in order to understand the role of peroxidases during the interaction between pistil and pollen, it is necessary to know their role in pollen and unpollinated pistils. Therefore, data on peroxidases before and after pollination are summarized and discussed in relation to the physiological processes which take place after pollination.

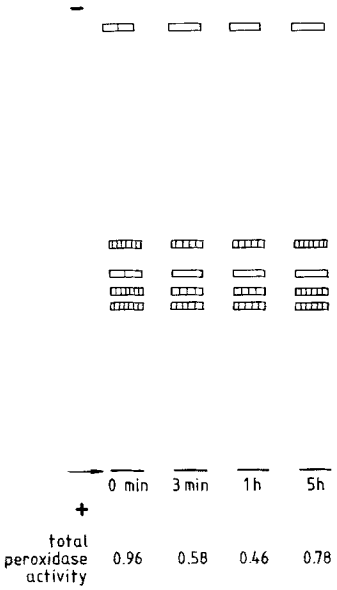

Fig. 1. Peroxidase activity (ΔA470/5 min/50 mg pollen) and peroxidase isoenzyme composition during germination and tube growth of *Nicotiana alata* pollen in vitro (Bredemeijer 1982 c)

2 Pollen peroxidases during in vitro growth

As has been suggested previously by Linskens and Kroh (1970) a fruitful approach to the problem of pollen tube growth in vivo is through the study of growth in vitro because it is possible to grow the pollen under controlled conditions and to investigate its reaction under the influence of different factors.

2.1 Peroxidase activity and peroxidase isoenzyme composition

Peroxidase activity has been reported in pollen of several hundreds of plant species (Poddubnaya-Arnoldi et al. 1961; Martin 1968; Brewbaker 1971; Malik and Mehan 1975). However, when compared to other tissues of the plant, pollen has a relatively low peroxidase activity as well as low isoenzyme polymorphism (Linskens 1966; Brewbaker 1971; Malik and Gupta 1976; Bredemeijer 1979). An extreme case is the pollen of *Oenothera organensis* which shows no peroxidase activity (Lewis et al. 1976; Stanley and Linskens 1974).

Several authors have described the occurrence of changes in peroxidase activity and the isoenzyme pattern during pollen germination and tube growth in vitro. In the pollen of *Nicotiana alata* the peroxidase activity declined sharply within 3 min following the dispersal of the pollen in the culture medium (Fig. 1). After reaching a minimum at 1 h peroxidase activity increased steadily during pollen tube elongation (Bredemeijer 1982 c). The alterations in total peroxidase activity are reflected by changes in the activities of individual isoenzymes already present in the ungerminated pollen (Fig. 1). In several other plant species, by contrast, the peroxidase isoenzyme patterns also exhibited qualitative changes during pollen tube growth (Malik and Gupta 1976; Chhabra et al. 1978b). Dashek et al. (1979) reported that both the cytoplasm and the wall of germinating *Lilium longiflorum* pollen contain peroxidase activity. The wall associated peroxidases were of two types, ionically- and covalently bound. The cytoplasmic peroxidase activity decreased considerably during the first 2 h of germination and increased over the next 6 h while the activity of the wall peroxidase increased continuously with time.

2.2 Cytochemical localization of pollen peroxidase

In order to understand the role of pollen peroxidase it is important to know both the biochemical behaviour of the enzyme and its histochemical distribution. The site of localization of peroxidase strongly influences its catalytic function because of differences in isoenzyme composition and availability of H-donors.

There have been several studies on the localization of peroxidase in germinating and nongerminating pollen (see Bredemeijer 1982 c). In *Lilium longiflorum* the peroxidase activity is distributed throughout the cytoplasm in the older regions of the pollen tubes, but not within the growing tip and the region immediately adjacent to it (Fig. 2; Dashek et al. 1979). By contrast, in *Pinus roxburghii* (Mehan and Malik 1976) and *Calotropis procera* (Malik and Mehan 1975) peroxidase was found to be distributed throughout the whole length of the pollen tubes. In addition, it was shown that the exine of the pollen wall (Martin 1968) and the pollen tube walls contained peroxidase (Dashek et al. 1979).

2.3 Effects of physiological treatments on pollen peroxidases

Several exogenous factors, such as growth hormones, antimetabolites and spectral quality of light, are known to affect not only pollen tube growth in vitro but also the synthesis and activity of peroxidase. In general, the addition of IAA and GA_3 to germination medium increased pollen tube growth and peroxidase activity, whereas antimetabolites such as acti-

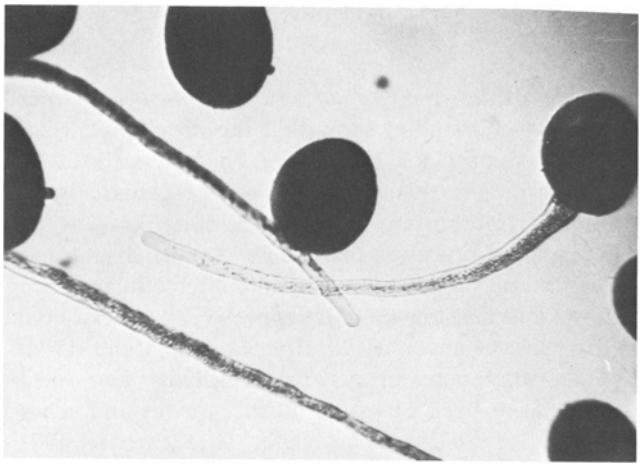

Fig. 2. Cytochemical localization of peroxidase in *Lilium longiflorum* pollen germinated for 4 h at 25 °C in vitro. (This photograph was kindly provided by Dr. W.V. Dashek)

nomycin D and cycloheximide showed inhibitory effects (Thapar et al. 1976; Chhabra et al. 1978 a, b).

In *Arachis hypogaea* and *Lathyrus odoratus* short exposure of pollen to red light increased pollen tube elongation and the activites of peroxidase and IAA oxidase respectively. This could be reversed by far-red (Sharma and Malik 1978; Chhabra et al. 1979), indicating that peroxidase is a phytochrome controlled enzyme. The effects of red and far-red light are probably specific phenomena. By contrast, other studies in *Pinus roxburghii* showed no effect of red and far-red light on pollen peroxidase activity in spite of growth stimulation by red light (Dhawan and Malik 1979).

3 Peroxidases in unpollinated pistils

Compared to the other parts of the plant, the pistil has a relatively low peroxidase activity and peroxidase isoenzyme composition, e.g. *Nicotiana tabacum* (Sheen 1969) and *Petunia axillaris* (van den Berg and Wijsman 1981). The high chlorogenic acid content of the flower parts should be responsible for this low peroxidase activity. The reports on pistil peroxidases concern in general the stigma and/or stylar parts, but not the ovary.

3.1 Stigma peroxidases

Peroxidase activity has been detected in stigma extracts or stigma diffusates of several species, but there exists differences (Martin 1968; Nasrallah et al. 1970; Bredemeijer and Blaas 1975). For instance, the stigmatic fluid of *Petunia hybrida* did not react in a cytochemical test for peroxidase (Herrero and Dickinson 1979). In *Nicotiana alata* the stigma contained higher peroxidase activity than that found in the style. In addition, qualitative and quantitative differences do exist between the isoenzyme patterns of stigma and style (Bredemeijer and Blaas 1975).

3.2 Style peroxidases

The occurrence of peroxidase in the stylar part of the pistil has been reported for only a few plant species (Brewbaker 1971; Bredemeijer and Blaas 1975). The most extensive study has been carried out with *Nicotiana alata,* i.e. in relation to incompatibility genotype, localization, development and senescence.

3.2.1 Incompatibility genotype and peroxidases. It has been suggested from investigations in *Nicotiana alata* that each S- (self-incompatibility) allele has its own specific stylar peroxidase isoenzyme bands (Pandey 1967). However, in studies with other S-genotypes, no relationship between the S-alleles and isoenzyme patterns could be found (Fig. 3; Bredemeijer and Blaas 1980). Therefore, it was concluded that the relationship, as found by Pandey (1967), is probably a nonspecific one, i.e. the peroxidases concerned are coded by closely linked genes instead of by the S-locus. The recent work by Labroche et al. (1983) with the same incompatibility system and leaf peroxidases in those genotypes previously used by Pandey (1967) showed that two peroxidase loci and the S-locus are located in the same chromosome and that one of the peroxidase loci is closely linked to the S-locus. Investigations in *Lycopersicon peruvianum* did not reveal any relation between the S-genotype and the peroxidase isoenzyme pattern of the leaves (Bredemeijer 1977 a).

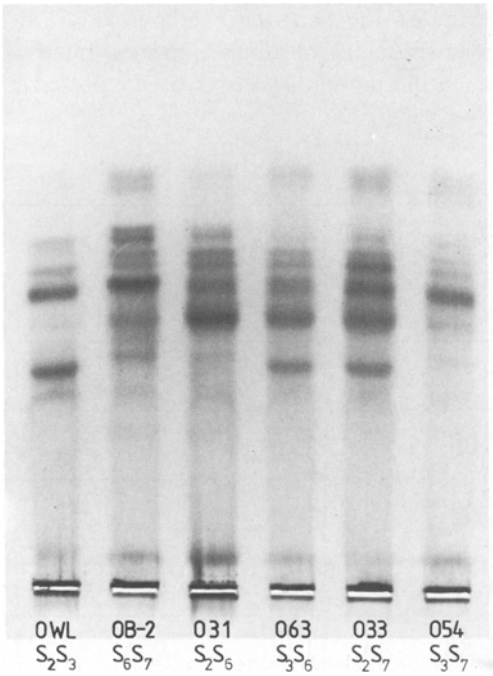

Fig. 3. Peroxidase isoenzymes in styles of the parent clones *OWL* and *OB-2* and their F1 progeny in *Nicotiana alata* (Bredemeijer and Blaas 1980)

3.2.2 Distribution of style peroxidases. From our previous work in *Nicotiana alata,* it was concluded that peroxidase activity is not equally distributed along the style (Bredemeijer and Blaas 1975). The activity decreases from stigma to basal end, the trend being reversed near the ovary. In addition, it was shown that the gradient in peroxidase activity is accompanied by changes in the contribution of particular isoenzymes (Fig. 4). It is known that gradients in peroxidase activity occur also in roots, epicotyles and tobacco pith (Lavee and Galston 1968; Birecka et al. 1972; Fielding and Hall 1978). Although their physiological role is still unknown, a correlation with growth patterns suggests a connection between the two phenomena (Lavee and Galston 1968).

Histochemical localization of peroxidase in transverse sections of *Nicotiana alata* styles revealed a strong reaction in the epidermis, the vascular system and the transmitting tissue and a weak reaction in the parenchymatous cortex cells (Bredemeijer and Blaas 1983). In general, the cell walls exhibit a considerable peroxidase activity. In situ extraction of the cell walls revealed that almost all the protoplast peroxidase isoenzymes occur also in the cell walls (Bredemeijer 1977).

The localization of peroxidase in the transmitting tissue is shown in Fig 5. Both cytoplasm and cell walls, and intercellular substance show a strong reaction which can be ascribed mainly to one tissue-specific isoenzyme (Bredemeijer 1979). In this connection it should be mentioned that Herrero and Dickinson (1979) demonstrated the occurrence of peroxidase in the intercellular substance of another species, i.e. *Petunia hybrida.* Furthermore, they observed the presence of acid phosphatase and esterase in this intercellular substance.

3.2.3 Peroxidase activity during development and senescence. Several studies show that the development and senescence of plant tissues are characterized by an increase in peroxidase activity and peroxidase isoenzyme polymorphism. In addition, it is known that during these processes peroxidase is partially released from cytoplasm to cell walls and intercellular spaces (Lavee and Galston 1968; Chappet 1970; Birecka et al. 1972; Birecka et al. 1979; Birecka and Miller 1974). Similar alterations in peroxidase activity and localization have been observed during growth and senescence of *Nicotiana alata* styles (Bredemeijer 1973, 1977). For instance, during style growth the peroxidase activity increases only slightly, but towards the end of the growth period the activity increases strongly due to the induction of a new isoenzyme.

Further, it has been shown that the ageing of detached *Nicotiana alata* flowers causes not only an increase in the activity of several stylar peroxidase isoenzymes but also the induction of a new isoenzyme (Bredemeijer 1974). This ageing-induced increase in peroxidase activity was genotype-dependent. Among the plants of an inbred progeny the induction of isoenzyme P-10 ranged from very weak to very strong (Bredemeijer 1982 a).

3.3 Ovary peroxidases

The peroxidase isoenzyme pattern of *Nicotiana alata* ovaries differs from that of the style both qualitatively and quantitatively (Fig. 4). The ovary has more isoenzyme forms of peroxidase and the activities of the individual isoenzymes exceed those of the corresponding isoenzymes in the style. In

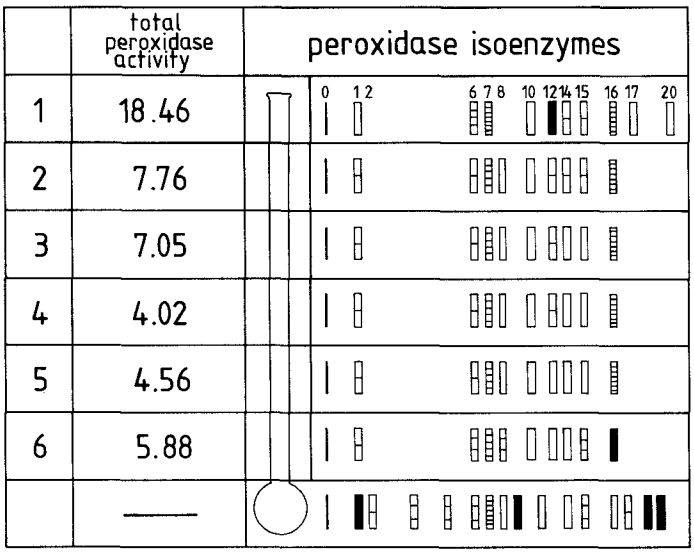

Fig. 4. Distribution of total peroxidase activity (ΔA334/5 min/g fresh weight) and peroxidase isoenzyme pattern in unpollinated pistils of *Nicotiana alata* (Bredemeijer and Blaas 1975)

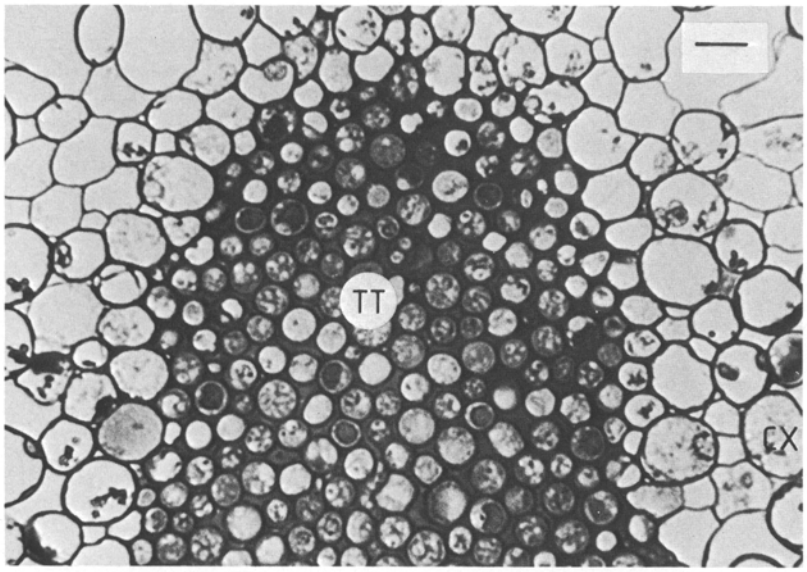

Fig. 5. Histochemical localization of peroxidase in a transverse section of the transmitting tissue (*TT*) and enveloping cortex (*CX*) of an unpollinated style of *Nicotiana alata*. Scale bar = 10 μm (Bredemeijer and Blaas 1983)

addition, the ovary contains two tissue-specific peroxidases and a number of peroxidases that also occur in the corolla but not in the unpollinated styles (Bredemeijer 1979).

4 Peroxidases in pollinated pistils

4.1 Peroxidase activity and peroxidase isoenzyme composition

Although pollination has no immediate effect on the total peroxidase activity of *Nicotiana alata* styles, the activity increases steadily during pollen tube growth through the style (Bredemeijer 1974; Bredemeijer and Blaas 1975). The increase in peroxidase activity is higher in cross-pollinated than in the self-pollinated styles. After the pollen tubes have reached the ovary, the increase in stylar peroxidase activity is enhanced strongly.

The increase in total peroxidase activity is reflected by a genotype-dependent increase in the activity of individual isoenzymes (Bredemeijer 1978, 1982a). The first effect of pollination was the appearance of some faint isoenzyme bands which correspond to the pollen peroxidases. Although the results suggest a higher activity of pollen peroxidases (P-3 and P-4 in Fig. 6) in certain sections of the style after incompatible pollination, the possibility of interference of the stylar isoenzymes with those of the pollen canot be excluded (Bredemeijer 1982c). During the growth of the pollen tubes through the style, the activity of many stylar peroxidase isoenzymes gradually increases (Fig. 6). Some of these isoenzymes (e.g. P-10) increase also during ageing of the unpollinated styles, but this effect was less pronounced.

A time course study of the distribution of peroxidase activity along the length of the style revealed that some isoenzymes in the cortex increase only following the passage of the pollen tubes, whereas others increase after penetration of the tubes into the style independently of growth rate or incompatibility reaction. The peroxidase activity in the transmitting tissue (P-12 in Fig. 6) decreases in the topmost part (i.e. 1 cm) of the style, whereas it increases in the lower parts (Bredemeijer and Blaas 1975; Bredemeijer 1979). Unlike several other enzymes (Roggen 1967) peroxidase did not show an increase in activity in that section of compatible pollinated styles where the pollen tubes have not yet grown. After syngamy, the activity of almost all the stylar peroxidases increases considerably. In addition, some new peroxidase isoenzymes are induced (Fig. 7). The final result is an isoenzyme pattern almost identical to that of the corolla (compare Bredemeijer 1974, 1979).

It is known that numerous biochemical processes take place during the progamic phase within the ovary and that, thereafter they intensify during the embryogenesis (Linskens 1973). Concerning the peroxidase activity during the progamic phase, no data are yet available on the possible changes. However, there are data on peroxidase in the ovary, after progamic phase, suggesting its increased activity. In *Nicotiana tabacum* capsule development is accompanied by an increase in the activity of several peroxidase isoenzymes (Sheen 1973). In the orchid *Encyclia tampensis* peroxidase activity increases linearly with fruit diameter and wet weight and is highest in the portion of the fruit which contains developing ovules. The peroxidase activity initiated in this fruit by pollination can be ascribed to a single isoenzyme (Alvarez 1968).

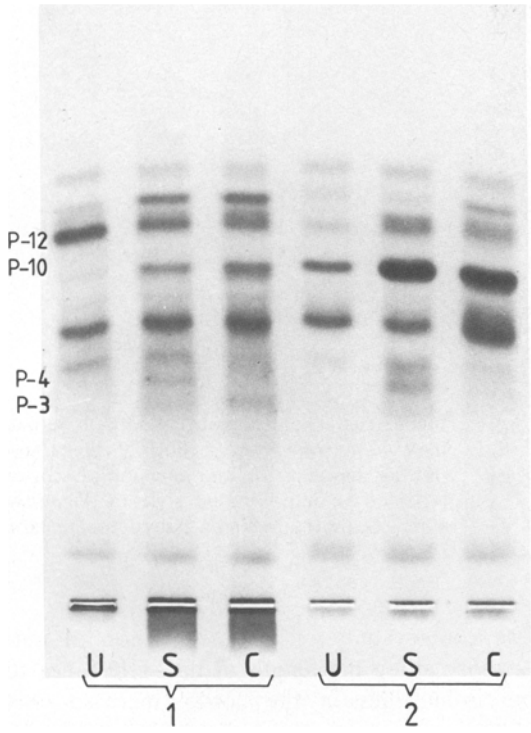

Fig. 6. Peroxidase isoenzyme patterns of the two top segments, *no. 1* (0–10 mm) and *no. 2* (10–20 mm) of unpollinated (*U*), self-pollinated (*S*) and cross-pollinated (*C*) styles of *Nicotiana alata* 4 days after pollination (Bredemeijer and Blaas 1975)

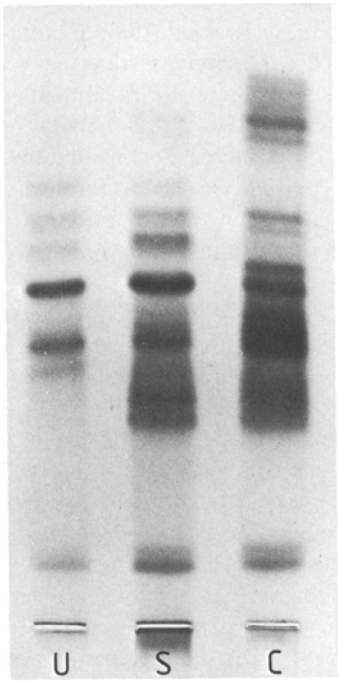

Fig. 7. Peroxidase isoenzyme patterns of unpollinated (*U*), self-pollinated (*S*) and cross-pollinated (*C*) styles of *Nicotiana alata* 7 days after pollination. The extract of cross-pollinated styles (*C*) was diluted three times (Bredemeijer 1974)

4.2 Histochemical localization of peroxidase activity

The sites of peroxidase localization in transverse sections of the cortex of pollinated *Nicotiana alata* styles are similar to those in unpollinated styles. However, the staining intensity of the cell walls and cytoplasm is higher in the pollinated styles than in the unpollinated styles (Bredemeijer and Blaas 1983). The increase in the amount of peroxidase in the cell walls after pollination was confirmed by in situ extraction of the walls. The same study revealed that four days after pollination the cell walls of cross-pollinated styles contained more peroxidase than those of self-pollinated styles. This may be due to differences in the degeneration of stylar cells observed following the passage of compatible and incompatible pollen tubes (Herrero and Dickinson 1979). On the other hand, differences in pollen tube number between compatible and incompatible crosses may also play a role.

In the transmitting tissue of *Nicotiana alata* styles, especially at sites with many pollen tubes, the peroxidase-positive intercellular substance is almost completely replaced by the tubes. The pollen tube walls are bipartite with a peroxidase-positive outer layer (pectocellulosic wall) and a peroxidase-negative inner layer (callosic wall). In addition, the cytoplasm reveals a strong peroxidase reaction (Fig. 8). The cytochemical localization of peroxidase at the light microscopic level indicated no differences between compatible and incompatible pollen tubes.

The distribution of peroxidase activity in the ovary after fertilization has been studied by Sheen (1973). Dissection of the parts of green capsules of *Nicotiana tabacum* showed a high peroxidase activity in the placenta and a lower activity in ovary wall and ovules. Histochemical studies indicated that peroxidase is localized in the epidermal layers of the placenta and ovule as well as in the vascular strands of the placenta.

A study by Alvarez (1968) on the localization of peroxidase during pollination-induced fruit development in the orchid *Encyclia tampensis* has shown that during the early stage of development, peroxidase occurred only directly around the vascular tissues of the ovary wall. At a later stage peroxidase appears to be randomly distributed throughout the parenchyma. In the sporogenous tissue peroxidase is absent during the early stages. However, after the ovules have reached maximum size, peroxidase was also observed in the ovule proper.

4.3 Effects of physiological treatments on style peroxidases

Bud-pollination, delayed self-pollination, temperature treatment, irradiation and growth hormones are known to affect pollen tube growth. Such treatments have been used to induce a temporary breakdown of the self-incompatibility character (for review, see De Nettancourt 1972). Several authors have suggested that the effects of such treatments are primarily exerted through their influence on enzymes (Townsend 1968; Hopper and Peloquin 1968; Pandey 1973). Therefore, studies have been carried out to establish whether peroxidase belongs to this category of enzymes.

4.3.1 Bud-pollination. The principle of self-compatibility following bud-pollination of self-incompatible species is based on the fact that the incompatibility substances are absent or not fully effective in developing styles

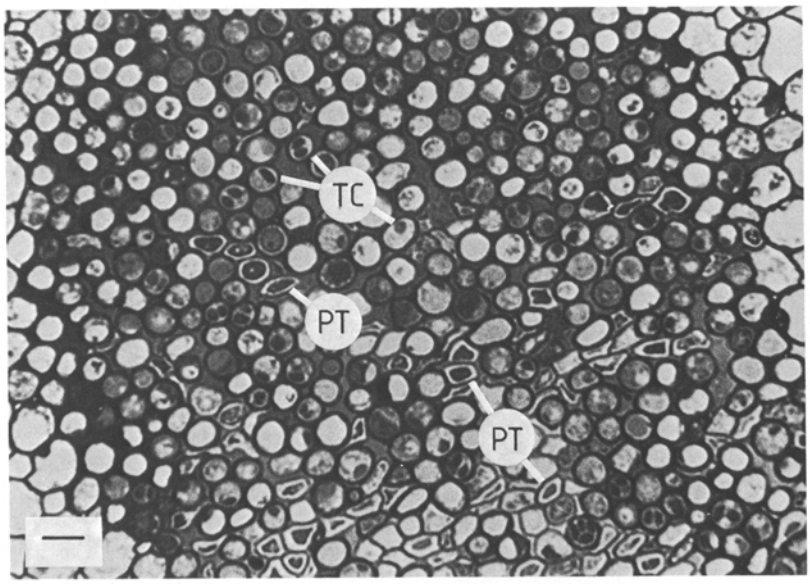

Fig. 8. Histochemical localization of peroxidase in the transmitting tissue of *Nicotiana alata* 4 days after compatible pollination. Scale bar = 10 μm. *PT* pollen tube; *TC* transmitting tissue cell (Bredemeijer and Blaas 1983)

(Linskens 1964). Among the developmental changes in the peroxidase isoenzyme pattern of the style of the *Nicotiana alata* clone OWL, two occurred almost simultaneously with the shift from compatibility to incompatibility. The first one, i.e. the induction of a specific peroxidase isoenzyme during the last phase of the growth of the style (Bredemeijer 1973), is probably not involved in the building up of the biochemical barrier controlling self-incompatibility. The activity of this isoenzyme decreased after anthesis, while the strength of pollen tube growth inhibition still increased.

The second developmental alteration was observed only in pollinated styles. The capacity of the style to induce isoenzyme P-10 after pollination arose at the same time as the shift from compatibility to incompatibility, within one day before anthesis. Afterwards, the induction of P-10, as well as the incompatibility reaction, became stronger. In conclusion, the P-10 induction seems to be positively correlated with the strength of the incompatibility reaction (Bredemeijer 1976).

4.3.2 Irradiation treatment. Irradiation of *Nicotiana alata* flowers with high doses of γ-rays (400-600 Krad) immediately before pollination resulted in the breakdown of the incompatibility reaction (Bredemeijer et al. 1981) confirming the previous results reported by Linskens et al. (1960) and Hopper and Peloquin (1968). In addition, there was a decrease in the number of compatible and incompatible pollen tubes and the callose staining of the tube walls. The latter indicates a decrease in callose deposition or a degradation of callose as suggested by Gilissen (1978).

The analysis of the peroxidase isoenzyme pattern of the irradiated styles revealed two effects, i.e. a gradual increase in the activity of certain cortical peroxidases during several days and an abrupt decrease of peroxidase in the transmitting tissue immediately after irradiation (Bredemeijer et al. 1981). The latter effect might cause the reduction of the number of pollen tubes and callose staining (see section 6.2.2).

4.3.3 Plant growth regulators. The growth substances, such as auxin, ethylene and gibberellic acid, alone or in combination, are known to induce qualitative and quantitative changes in the peroxidase isoenzyme patterns of various plant tissues (e.g. Ockerse and Mumford 1973; Henry and Jordan 1977; Gaspar et al. 1978). Treatment of *Nicotiana alata* pistils with IAA or ethylene enhanced the activity of several peroxidase isoenzymes in the style (Bredemeijer 1982a,b). It is likely that the effect of IAA is mediated via ethylene as both hormones have almost similar effects on the peroxidase isoenzyme pattern. Moreover, the increase in peroxidase activity in IAA-treated pistils is preceded by a surge of ethylene production.

5 Mechanism of peroxidase induction after pollination

The pollination-induced alterations in the peroxidase isoenzyme pattern of *Nicotiana alata* styles are comparable to those induced by treatment with IAA and ethylene (section 4.3.3; Bredemeijer 1982a). It is known that the concentrations of these growth hormones in the pistil increase during penetration and growth of the pollen tubes and after syngamy (Muir 1942, 1947; Lund 1956; Nichols 1977; Bredemeijer 1982b). Since auxin stimulates the production of ethylene (Hall and Forsyth 1967), it is likely that the effect of pollination

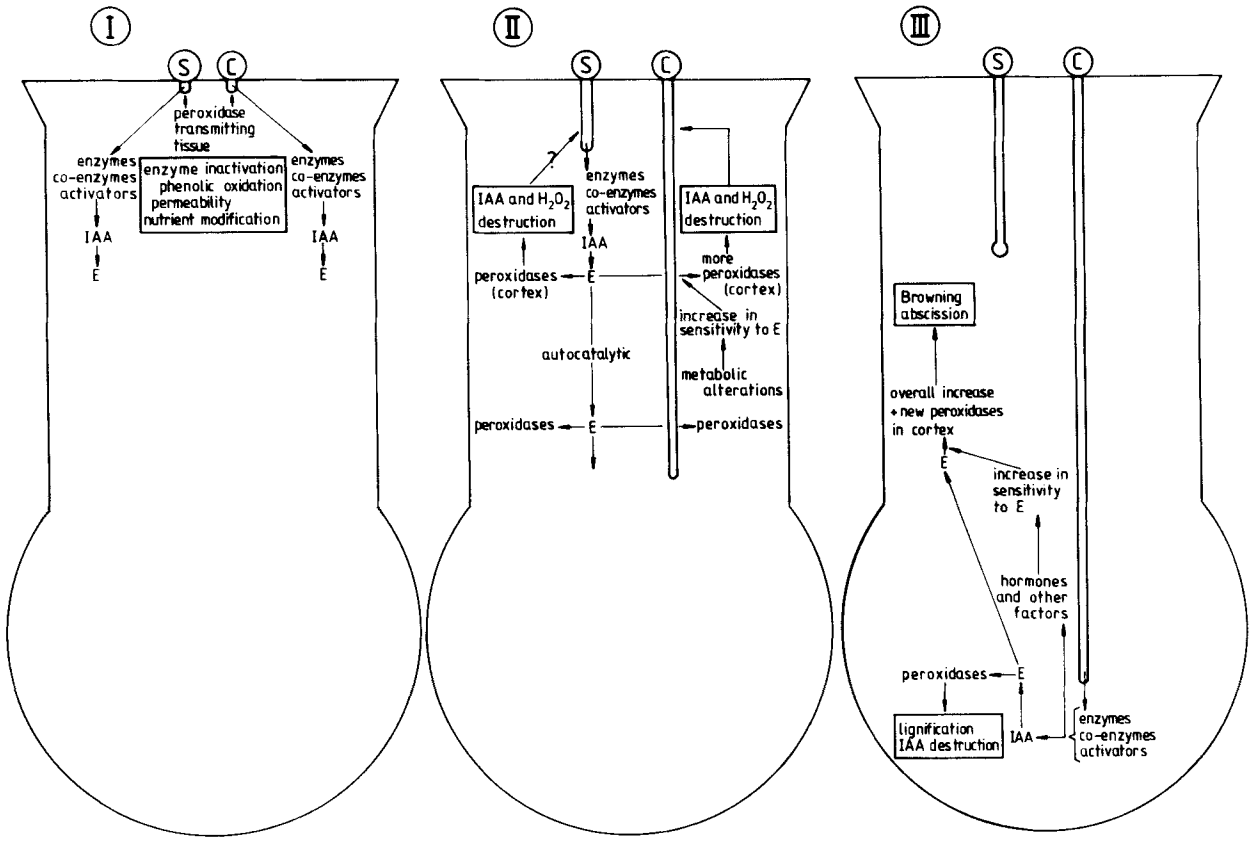

Fig. 9. A schematic presentation showing the probable reactions leading to the induction of peroxidase isoenzymes and their possible roles in pollinated pistils. The scheme is based on the results obtained in *Nicotiana alata* and *Nicotiana tabacum*. *S* self-incompatible pollen; *C* cross-compatible pollen; *E* ethylene

on pistil peroxidases is mediated via auxin-induced ethylene (Fig. 9; Bredemeijer 1982a). The increase in auxin content, in turn, is caused by substances released from pollen tubes. These substances convert the bound IAA in the pistil to free IAA (Muir 1947) or stimulate the synthesis of IAA (Lund 1956).

The ethylene evolution is, once started, autocatalytic. Each cell triggers the neighbouring one to produce ethylene (Burg and Dijkman 1967). This process is reflected in *Nicotiana alata* styles by the induction of peroxidase isoenzymes, e.g. P-10. There is no evidence at the moment that this isoenzyme is involved in ethylene synthesis as has been proposed for other peroxidases by Yang (1967) and Mapson and Wardale (1971). Genotypes showing high P-10 activity did not produce more ethylene than genotypes with a low P-10 activity (Bredemeijer 1982b).

The mechanism of peroxidase induction in pollinated styles may be the same as that in aged unpollinated styles. In unpollinated styles the auxin and ethylene concentrations increase, but at a much lower rate than in the pollinated styles (Lund 1956; Bredemeijer 1982b).

Apparently, the pollination-induced acceleration of stylar ageing is mediated via an increased ethylene production that is reflected by an increase in peroxidase activity. The fact that infiltration of style segments with solutions of actinomycin D or cycloheximide strongly reduced the increase in activity of ageing-induced peroxidases (Bredemeijer, unpublished results) suggests that the increase in activity is due to a de novo synthesis.

Undoubtedly, several other factors in addition to ethylene are involved in the induction of pistil peroxidases. The variation in the ethylene-induced peroxidase isoenzyme activity among different genotypes and the different responses of individual isoenzymes (Bredemeijer 1982a,b) indicate that the regulation of peroxidase induction by ethylene is complex. It is likely that differences in sensitivity to ethylene play an important role. Ethylene production in styles with a weak peroxidase induction is approximately the same as that in styles with a strong peroxidase induction. However, the concentration of ethylene necessary to induce, for example, isoenzyme P-10, is significantly greater in the former type. Furthermore, the fact that some ethylene-

induced peroxidases increase in pollinated styles only after the passage of the pollen tubes, while other ethylene-induced peroxidases are enhanced independently of pollen tube length, may suggest a change in sensitivity to ethylene in stylar tissue after passage of the tubes (Fig. 9).

The differences in sensitivity to ethylene can be the result of an interaction between factors, such as hormones, carbohydrate reserves and osmotic concentration of the tissue (for a review, see Mayak and Halevy 1980). It is, in this context, interesting to mention that application of a high molarity solution of saccharose to the stigma causes an increase of the peroxidase isoenzymes in the style comparable to that found after pollination and syngamy (Bredemeijer, unpublished results).

The fact that young flowers are not responsive to ethylene, unlike mature flowers, may account for the influence of the developmental stage on the pollination-induced increase in peroxidase activity in the style (see section 4.3.1). The responding system becomes, in general, more sensitive to ethylene as the flower organs mature (Mayak and Halevy 1980). This tendency is reflected by the induction of certain peroxidases in the style (Bredemeijer 1976).

6 The role of peroxidases in pistil-pollen interactions

Despite the extensive data on pollen and pistil peroxidases, little is known about the physiological role of these peroxidases. Firstly, this is due to the lack of knowledge not only on the site of the localization of specific peroxidase isoenzymes, but also about the distribution and relative amounts of substrates (peroxides) and hydrogen donors. Secondly, the physiological role of plant peroxidases, in general, is still obscure. So far, only one style peroxidase isoenzyme has been localized precisely, i.e. the transmitting tissue-specific peroxidase in *Nicotiana alata* (Bredemeijer 1979; Bredemeijer and Blaas 1983). The other peroxidases in the pollen and style of *Nicotiana alata* are not tissue-specific, indicating their non-specific roles respectively in pollen and pistil functions.

6.1 Pollen peroxidases

It has been difficult to speculate on the role of pollen peroxidases in vivo from the isoenzyme studies performed on pollinated pistils (section 4.1). Therefore, several authors have attempted to explain the role of pollen peroxidases from data obtained from in vitro studies (section 2).

In the species investigated, the peroxidase activity of the pollen initially decreases after dispersion in the culture medium. Subsequently, the activity increases with elongation of the pollen tubes. The rapid decrease in peroxidase activity after dispersion of pollen in a culture medium is probably caused by the release of the enzyme from the pollen into the medium, a phenomenon which has been reported for several plant species (Martin 1968; Malik and Gupta 1976; Ashford and Knox 1980; Dashek 1982). It is known that several other enzymes, such as esterase, acid phosphatase and amylase, are also released from germinating pollen in vitro and in vivo (Stanley and Linskens 1965; Heslop Harrison 1975; Ashford and Knox 1980). Mäkinen and Brewbaker (1967) proposed that the enzymes released from the pollen grains metabolize the compounds of stigmatic tissues to products which influence the early growth of pollen tubes and possibly germination. The continuous diffusion and action of pollen enzymes on stylar tissue might serve as a source of nutrients for the elongating tubes.

The possible interactions between the peroxidases released from pollen and compounds in stigma/style have been discussed recently (Bredemeijer 1982c). To briefly conclude, the function of the released peroxidases may be related to the phenolic substances in the stigma and stigmatic exudate. On the one hand, the phenolic compounds, such as caffeic acid, chlorogenic acid and cinnamic acid, which are present in stigmatic exudates, are hydrogen donors for peroxidase. On the other hand, the phenolic compounds may stimulate or inhibit the IAA oxidase activity of the released peroxidase, and thus influence the growth processes. Chhabra et al. (1979) proposed a role of the released pollen peroxidase in the degradation of microbiologically produced exogenous IAA on the stigma.

The increase in peroxidase activity in both cytoplasm and cell walls of elongating pollen tubes may be related to cell wall formation. The peroxidases could function in the hydroxylation of proline during the wall extension (Singh et al. 1976), in the regulation of the level of endogenous IAA to an extent essential for the elongation of the tubes (Pandhol and Malik 1978) or in the cross-linking of cell wall macromolecules by converting ferulic to diferulic acid, which can act as a hemicellulose cross-link (review Gaspar et al. 1982).

In addition to its role in cell wall formation pollen peroxidase has been proposed to be involved in a number of other processes related to ascorbic acid metabolism (Malik and Mehan 1975; Chhabra et al. 1978a) and IAA metabolism, the latter by its IAA oxidase activity. For instance, Malik and Chhabra (1978) have demonstrated that the pollen germination and early tube growth is regulated by IAA, but during the later phase of tube growth, the endogenous level of IAA must be destroyed. The stimulation of the IAA oxidase has been proposed to perform this function. Blue light or ethylene were found to destroy the IAA

by stimulating IAA oxidase (Chhabra and Malik 1978). This implies that the ethylene which is produced by the pistil may regulate pollen tube growth in vivo.

The wall fraction of peroxidase may protect the pollen tubes against an inhibiting concentration of IAA which is produced in pollinated pistils.

6.2 Stigma and style peroxidases

The occurrence of many peroxidase isoenzymes in the stigma and style, their differential distribution and response to pollination indicate that the various peroxidase isoenzymes or groups of isoenzymes perform different catalytic functions. In order to discuss the role of style peroxidases, it is necessary to distinguish between the isoenzymes in the cortex including vascular traces and those in the transmitting tissue.

6.2.1 Cortex. Among the cortical peroxidases in *Nicotiana alata* styles three groups can be distinguished based on their reaction to pollination.

Group 1. This group consists of only one isoenzyme (P-16) and is characterized by a decrease in activity which is equal to that in the unpollinated style (Bredemeijer 1974). Since the activity of this isoenzyme rises sharply at the end of the growth period it is probably involved in growth regulation by means of IAA destruction (see review in Gaspar et al. 1982). It is known that there exists an inverse correlation between the peroxidase activity and the growth potential (Lavee and Galston 1968; Chappet 1970).

A considerable proportion of isoenzyme P-16 occurs in the cell walls (Bredemeijer 1977) and therefore a role in lignification or overall stiffening of the walls (Fry 1979) also seems possible.

Group 2. These peroxidases increase in activity not only after pollination, but also during the ageing of unpollinated styles. The pollination accelerates only the ageing-induced increase (Fig. 9 II). In spite of the fact that peroxidase is considered as one of the most reliable indicators of senescence, no conclusive evidence of a cause-effect relation between the two is yet available (Birecka et al. 1979). According to these authors, the function of peroxidase isoenzymes whose activities increase with senescence, might be related to the elimination of hydrogen peroxide the production of which increases also with the senescence. Peroxidase might also have a stabilizing effect on chlorophyll. Thus, the increase in peroxidase activity would represent an induced protective reaction, perhaps delaying senescence (Birecka et al. 1979). It is not yet known whether the stylar peroxidases perform such a role. In any case, the growth rate of compatible pollen tubes in aged *Nicotiana alata* flowers is the same as compared to that in flowers pollinated at anthesis (Bredemeijer 1977) in spite of great differences in peroxidase activity of the styles. By contrast, the growth rate of incompatible pollen tubes retarded additionally in the aged flowers depending on the genotype. The positive correlation between this retardation and the activity of isoenzyme P-10 during development and senescence in clone OWL (see section 4.3.1) is certainly not a general phenomenon, but exists only in genotypes characterized by a relatively weak incompatibility reaction (Bredemeijer, unpublished results). Probably the pollination-induced peroxidase activity is only one of the physiological characters of a style which are known to contribute to the incompatibility reaction (Linskens 1977).

Group 3. These are only induced when pollen tubes reach the ovary (Fig. 9 III). The induction of several new peroxidases coincides with an overall increase in the activity of the pre-existing ones (Fig. 7). Similar observations were made in the corolla of pollinated *Phalaenopsis amabilis* flowers (Trippi and Tran Thanh Van 1971). The overall increase in peroxidase activity in the style and corolla is due to the activation of the ovary which is accompanied by changes in the growth hormone balance. These alterations may cause not only the increase in ethylene production but also a greater sensitivity of the tissue to ethylene (see section 5). This in turn, causes the senescence of the tissue as well as the increase in peroxidase activity. After syngamy the style, without further function, becomes brown and finally abscisses. As peroxidases are known to be involved in browning and abscission via phenolic oxidation and auxin destruction (Henry et al. 1974) respectively, it seems likely that the style peroxidases induced after syngamy perform such roles.

6.2.2 Transmitting tissue. The peroxidase activity in the transmitting tissue of *Nicotiana alata* styles is probably correlated closely with the special physiological functions of this tissue, i.e. nutrition of the pollen tubes and incompatibility reaction (Linskens and Kroh 1970; Vasil 1974). The reduction of the peroxidase activity in the transmitting tissue by irradiation treatment (section 4.3.2) is accompanied by a decrease in callose staining of the pollen tube walls suggesting a role of this style peroxidase in tube wall formation (Bredemeijer et al. 1981). This assumption is supported by the fact that horseradish peroxidase, at relatively low concentrations, stimulates the growth of pollen tubes in vitro (Bredemeijer 1975). It should be mentioned here, however, that high concentrations of peroxidase inhibits in vitro growth and causes thinning of the tube walls which

results in the opening of the tubes and subsequent release of their contents.

On the one hand, it is possible that the presence of peroxidase in cell walls and intercellular substance of the transmitting tissue is incidental and related to membrane functions as suggested for peroxidases in other plant tissues (De Jong 1966, 1967). Thus, the peroxidase may play, through its influence on the permeability of the stylar cell membranes, an important role in the nutrition of the pollen tubes. On the other hand, it is also possible that the secretion of peroxidase occurs to play a specific role in the intercellular substance. One can think, in this connection, of the modification of nutrients, the inactivation of enzymes secreted by the pollen tubes (Mäkinen and Brewbaker 1967) and the oxidation of phenolic compounds originating from pollen (Stanley and Linskens 1965) or pistil (Sheen 1969). It should be noted here that the peroxidase in the transmitting tissue is able to oxidize the naturally occurring phenolic compounds, caffeic- and chlorogenic acid (Bredemeijer, unpublished results). Furthermore, the peroxidase in the intercellular substance may increase the permeability of the membranes of the pollen tubes similar to that observed after treatment of yeast cells with peroxidase (Niedermeyer 1975).

It is premature to speculate here on the way in which the peroxidase in the intercellular substance may be involved in the regulation of pollen tube growth. Many enzymes, substrates and other compounds released from both pollen and style (Linskens 1968a; Bredemeijer and Blaas 1983) can interact in the intercellular substance. The possible interactions are, however, numerous. The interplay of various phenolic compounds and enzymes, such as peroxidase and polyphenol oxidase, which are involved in their metabolism (Sheen 1969; Bredemeijer 1979), deserves special further attention.

6.3 Peroxidases in the ovary

The pollination-induced increase in peroxidase activity in the developing fruit of the orchid *Encyclia tampensis* reflects the continued production of auxin by the pollen or the tissues of the column (Alvarez 1968). The same author proposed that this peroxidase activity may play a role in the inactivation of auxin and in lignin biosynthesis. The latter is supported by the fact that the highest peroxidase activity in the mature fruit is localized in the ovule, a structure which in its final phases of development undergoes lignification. As concerned the inactivation of auxin, Sheen (1973) proposed a similar function for peroxidase during capsule maturation in *Nicotiana tabacum* because this process marks a decline in auxin. In addition, the capsule peroxidase should perform a role in ethylene production (Fig. 9 III).

7 Conclusions

This review on the role of peroxidases in pistil-pollen interactions presents evidence that the majority of the peroxidase isoenzymes in the pistil are involved in general processes related to growth, development and senescence of the pistil. The influence of these peroxidases, which are not tissue-specific, on pollen tube growth seems to be indirect. Probably only the tissue-specific peroxidase in the transmitting tissue of the stigma and style plays a direct role in the interaction between pistil and pollen tubes.

The pollen peroxidases are probably involved in the regulation of the growth of pollen tubes and in tube wall formation. The peroxidases released from pollen may play a role in the pistil-pollen interaction related to the phenolic compounds in the stigma and its exudate. With respect to the pollen peroxidases, more attention has to be given to their activity and localization in vivo. Although the use of in vitro grown pollen tubes has several advantages (Rosen 1968; Linskens and Kroh 1970), a knowledge of the pollen peroxidase activity, especially in the wall fraction, in the presence of substances normally available in the pistil, is indispensible. The problems related to the determination of the activity and localization of pollen peroxidases in vivo may be overcome by using a localization procedure based on the differences in temperature sensitivity of different isoenzymes and by using lily styles because in this species pollen tubes can be easlily removed from the hollow style canal (Bredemeijer 1982c).

Another problem to solve is the distribution of the cortical peroxidase isoenzymes with respect to parenchymatous cells, vascular traces and transition zone between cortex and transmitting tissue. In addition, the localization and relative amounts of substrates, hydrogen donors, inhibitors and activators which determine the activity of the available peroxidase have to be determined. Particularly, the phenolic compounds in the pistil (Sheen 1969, 1973; Martin 1970) may play an important role. These compounds may function not only as hydrogen donors for peroxidase, but also as inhibitors or activators, depending on their nature (Shinshi and Noguchi 1975; Jaeger-Wunderer 1980).

Finally, boron and calcium, which have a remarkable effect on pollen germination and tube growth (review in Linskens and Kroh 1970), may exert their effects by modifying the amount of peroxidase attached to cell walls, as suggested by Parish (1969) for other plant tissues.

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