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Review

Callose and determination of pistil viability and incompatibility

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Summary. Callose provides a useful phenotypic bioassay in plant breeding to determine: incompatibility system; gametophytic competition; and stigma and ovule viability. Callose appearance in ovules may be associated with senescence, and used to determine the effective pollination period. In incompatible matings, callose formation is specific and related to rejection phenomena. The stigma callose response is induced by informational molecules carried by the germinal line, i.e. self or interspecific pollen, but not by the somatic line. Several methods of visualizing callose are reviewed. The role of callose in pollen-stigma interactions has many analogies with host-parasite interactions, and a model is proposed based on relationships between callose, boron and inhibitor (phytoalexin-like) synthesis. The callose response provides a useful tool for the biotechnology of seed production.

Key words: Callose response – Ovule viability – Effective pollination period – Pollen-stigma recognition – Host-parasite interactions

Introduction

Callose accumulation in pollen and pistil provides plant breeders with a useful phenotypic bioassay for the rapid diagnosis of pollination. This is especially useful (a) at the site of rejection of pollen tubes in incompatibility phenomena (Linskens and Esser 1957); (b) for estimates of the dynamics of pollen tube growth in gametophytic competition (Mulcahy 1975); (c) in estimating the viability of the pistil in determining the effective pollination period (Anvari and Stösser 1978).

Callose, a cell wall polysaccharide composed generally of 1,3 β-glucans, can be localized by the decolorized aniline blue fluorescence (ABF) method (Currier 1957; Linskens and Esser 1957). In several differentiation programmes in plant tissues, callose is rapidly synthesized, especially after wounding and during plant host/parasite interactions, particularly during pollination (reviews by Aist 1976; Bushnell 1979; Heslop-Harrison 1978). The callose response during pollination may be highly specific, occurring in stigma cells in contact with incompatible, but not with compatible pollen (Fig. 1) in genera such as *Brassica, Raphanus, Cosmos* and *Helianthus* which have well-developed sporophytic self-incompatibility systems (review by de Nettancourt 1977). Wall-held pollen proteins elicit the response (Heslop-Harrison et al. 1974, 1975) and its specificity has been explored using cell surface probes (Kerhoas et al. 1983). Recently, Sood et al. (1982) have claimed that the response may be induced not only by pollen grains, but by macerates of somatic tissues.

It is timely to review the evidence for the callose response, both as an indicator of biocommunication between pollen and stigma, and as a diagnostic tool for rapid assessment of the nature of a pollination in the biotechnology of seed production.

Callose and pistil viability calendar

In plant breeding, it is often not possible to determine the period of stigma receptivity and ovule viability without seed set trials which are time-consuming and costly. The ABF method provides a tool for determining these parameters. The technique is based on the appearance of callose in the pistil tissues concerned, which marks the end of the period of receptivity or viability (Fig. 2 A).

The close of the effective pollination period may be marked by the spread of callose through the cell walls

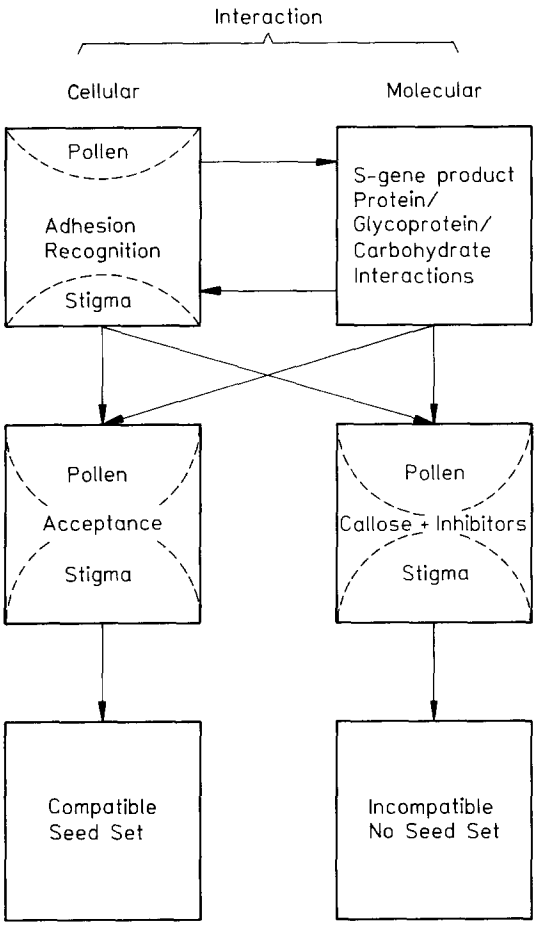

Fig. 1. Induction of callose during pollen-pistil interactions following compatible (*left*) or incompatible pollination (*right*). In (1) the *S*-gene products interact to initiate the acceptance or rejection responses (2). In (3) rejection is indicated by the presence of callose in either or both partners, and no seed set is a consequence

of the stigma, e.g. in *Brassica* (Dickinson and Lewis 1975) and in avocado, *Persea* (Sedgley 1979). Callose is absent from avocado stigmas in the receptive female phase, but accumulates in the stigma cell walls during the subsequent male phase of the dichogamous flowers.

Callose has been rarely noted in the style. An exception is the pome and stone fruits, where callose has been observed in the peripheral cells of the transmitting tissue (Heslop-Harrison 1976; Raff et al. 1983). There are no observations of the fate of this layer in non-receptive styles. It may act like an endodermis in controlling water loss or exchange of substances between the pathway of the pollen tubes and other stylar tissues.

In ovules, the loss of viability for pollen tubes is indicated by the appearance of callose and its progressive spread across the cells of unpollinated ageing ovules. This has been observed using the ABF method in avocado (Tomer and Gottreich 1975), in various species of *Prunus,* including cherry and peach (Anvari and Stösser 1978; Martinez-Tellez and Crossa-Raynaud 1982) and apple, *Malus* (Anvari and Stösser 1981). Callose is known to occlude nucellar cells of *Oenothera,* forming a 'crown' at the chalazal end of the ovule (Jalouzot 1970), and has been recorded in cell walls of the hypostase, at the same end of the ovule in *Antirrhinum* (Rodkiewicz 1967), and *Rhododendron* (Williams et al. 1982). It is not known whether callose spreads from this focal point or not during loss of viability, but such an interpretation is suggested by the photographs of Martinez-Tellez and Crossa-Raynaud (1982).

The presence of callose in cell walls has been associated with the presence of hydrolytic enzymes, e.g. in pollen of *Chlorophytum, Hyacinthus, Impatiens, Tradescantia* (Gorska-Brylass 1967), *Dactylorchis* (Heslop-Harrison 1968) and *Helianthus* (Vithanage and Knox 1979). One explanation for the presence of callose in the various tissues of the ovule, especially the hypostase (whose function is not known, review by Kapil and Tiwari 1978) is that it may be associated with ovule senescence through control of tissue autolysis by hydrolytic enzymes.

We may conclude that callose provides an indicator of the pistil viability calendar, both for the stigma cells, and for the ovule. It is likely that the callose is produced as a prelude to pistil senescence.

Callose in mature pollen

Callose has only rarely been recorded in the mature ungerminated pollen of angiosperms (Fig. 2 A). In sunflower pollen, positive ABF, indicating callose, occurs in the inner nexine layer of the exine, the outermost wall layer (Vithanage and Knox 1979). In the pollen of *Populus,* it occurs in the intine of some but not all grains (Ashford and Knox 1980). In gymnosperm pollen, the situation is quite different. Callose is present regularly in the intine of *Pinus* pollen (Waterkeyn 1964) and forms callose grains similar in size and appearance to starch grains in *Encephalartos, Gingko, Cunninghamia, Juniperus, Abies* and *Pinus* (Baker and Baker 1983). Callose in pollen walls may prevent modification of the water status of the grains at pollination.

Callose in germinating pollen

During pollen hydration and germination, callose is laid down in several wall sites (Fig. 2 A). Before germination, callose frequently adcrusts to the intine at the aperture nearest to the stigma surface. In the pollen tube, it forms the innermost zone of the bilayered wall and also the callose plugs. These latter structures are

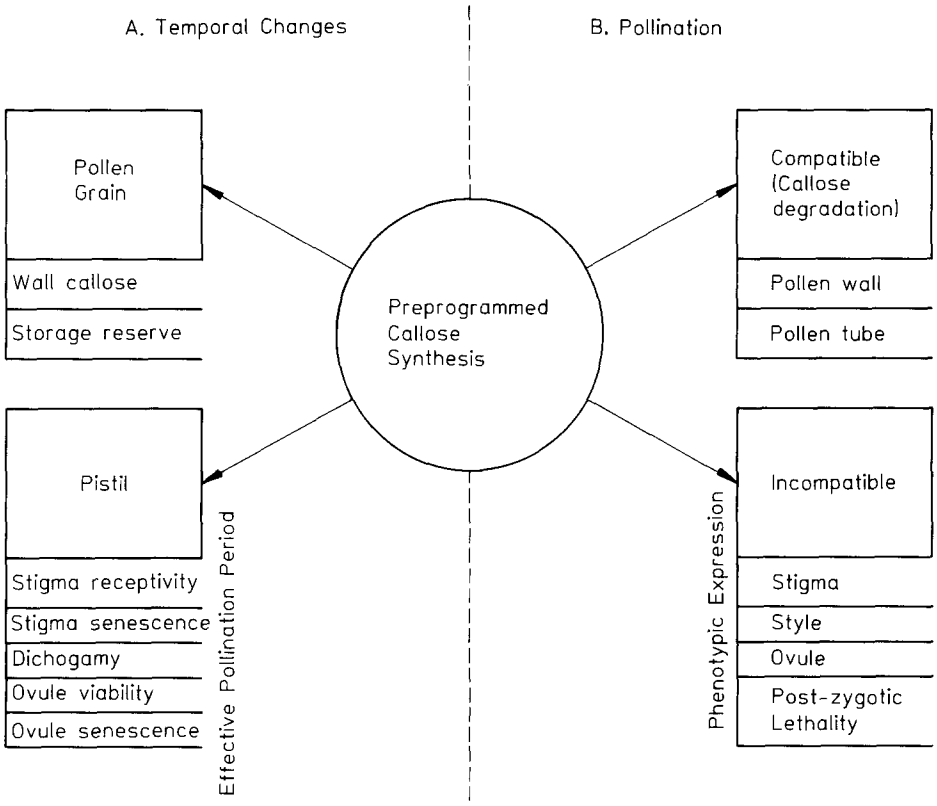

Fig. 2. Callose as a diagnostic tool in pollen and pistil before and during fertilization. On *left*, temporal changes are listed, including stigma receptivity and ovule viability. On *right*, presence of callose may indicate type of pollination and site of rejection in incompatible pollinations

localized wall thickenings which may partially or completely wall off the living cytoplasm of the tube tip, which is normally free of callose, e.g. in *Lycopersicon* (Cresti et al. 1977). Only if tube growth is arrested does callose deposition occur at the tube tip.

The site of callose and the form of the plug are remarkable. First of all, a ring of callose appears outside the plasma membrane, which increases in size until it completely fills the tube and induces autolysis (Cresti et al. 1977). A holocrine secretion mechanism may lead to enzyme activation and callose plug formation. We consider that this result may be induced by physical stress related to the decreased turgor pressure during tube elongation. Turgor pressure in the tube may be maintained by the formation of a series of plugs. Pressure is needed for tube penetration into the pistil, which provides a virtual mechanical facilitation pathway for the tubes (Dumas et al. 1982; Knox 1983). Physical stress changes are known to induce callose formation (review by Aist 1976) and a new plug would restore pressure. The result is a succession of plugs, resembling a ladder, along the elongating pollen tubes.

In tricellular types of pollen, plugs form early in tube development: e.g. in *Brassica*, the first plug is formed when the tube tip makes contact with the stigma surface, approximately 2 min after germination (Kerhoas et al. 1983). In bicellular types of pollen, their formation may be delayed: e.g. in *Petunia*, callose plugs were not visible by the ABF method until approximately 11 h after pollination (Mulcahy and Mulcahy 1983). Callose plugs developed at about the same time in in vitro culture – just prior to cessation of growth. The ABF method provides an essential tool in the study of plug formation and tube growth.

Since the pollen tube is lined throughout most of its length with callose, it is rendered relatively impermeable (Reynolds and Dashek 1976). Picton and Steer (1982) suggest that the presence of callose would limit the entry of calcium ions to the tip region, where callose is usually absent. In this way, the maintenance of tube polarity and direction of growth is ensured. This concept may also indicate that any *S*-gene specified receptors for the control of self-incompatibility must be located at the living tube tip.

The callose response

(a) Self-incompatible matings

The callose response may differ between the two main types of self-incompatibility: occurring in both pollen

and stigma in sporophytic systems; and only in the pollen and pollen tube in gametophytic systems (Fig. 2 B).

In sporophytic systems, recognition and self pollen rejection take place entirely on the stigma. The phenotype of rejection includes the deposition of lenticules (adcrustations) of callose in the periplasm of the stigma papillae adjacent to the incompatible pollen and within the germinating pollen grains and tubes, especially at the tips of rejected tubes (Dickinson and Lewis 1973 a; Heslop-Harrison et al. 1973, 1974; Knox 1973). The reaction of both partners is apparently simultaneous and occurs early in the recognition processes (Heslop-Harrison 1978). In sigmas of *Brassica,* callose may appear within minutes of pollen contact, before any erosion of the stigma cuticle (Heslop-Harrison et al. 1975), and may even occur prior to any appearance of callose in the pollen tube (Roberts et al. 1979) although this has recently been shown to depend on the strength of the self-incompatibility alleles (Kerhoas et al. 1983). In compatible pollinations, little or no callose is formed, and the pollen tube fuses with the stigma cell wall in a manner analogous to fungal pathogens (Bushnell 1979).

Callose adcrustation takes place in quite a different way to primary wall formation (Preston 1979). Ultrastructural features of callose lenticule formation in stigmas of *Raphanus* are given by Dickinson and Lewis (1975), and the processes are remarkably similar to certain host-parasite interactions (Bushnell 1979).

In gametophytic systems, rejection generally occurs within the stylar transmitting tissue, although in Gramineae and Onagraceae the site may be within the stigma (review by de Nettancourt 1977). In grasses, callose is formed within the incompatible tubes, as a wall lining, plugs and occluding the tip (Shivanna and Heslop-Harrison 1978). In other systems where arrest occurs within the style, the cytological appearance of rejected tubes does not differ until after arrest. Callose may accumulate in the tube tips, which may burst, perhaps in premature discharge of their gametes (Cresti et al. 1977). There are no reports of stigma or stylar callose responses in these systems.

From these observations, we can conclude:

– the stigma callose response in sporophytic systems is specifically induced by self but not compatible pollen, so that callose production is a response to *S*-gene expression.

– the elicitor of the stigma response is present on the pollen grain surface and is probably a protein.

– the response in pollen tubes following a self pollination is also specific but callose deposition may follow tube arrest.

(b) Compatible matings

In most compatible systems, the ABF method permits quantitation of tube growth, both in terms of the distance penetrated, and the relative numbers of pollen tubes in different regions of the pistil (see methods of Bernhardt et al. 1980 and Williams et al. 1983).

(c) Interspecific matings

Callose responses in both stigma and pollen are a feature of this type of incompatibility: matings between closely-related species or genera (not to be confused with incongruity and other types of wide hybridization, Hogenboom 1979). These responses occur in families with sporophytically determined self-incompatibility systems: e.g. Compositae (Vithanage and Knox 1977) and Cruciferae (Heslop-Harrison et al., unpublished data; Kerhoas et al. 1983). The response is so striking that it provides evidence for a dialogue between pollen and stigma. The strength of the callose response in the stigmas varied according to the taxonomic distance of the pollen species from the stigma. Pollen from genera in other families generally failed to elicit a response, since it usually failed to hydrate on the stigma (Vithanage and Knox 1977).

In interspecific matings between *Populus deltoides* and *P. alba,* which are incompatible, the tubes are arrested in the style. There is no callose response in the stigma cells, but the *P. alba* pollen tubes contain far more callose than their selfed *P. deltoides* counterparts (Knox et al. 1972). However, a characteristic feature of the arrested *P. alba* tube tips is that they are occluded with callose, while adjacent stylar cells show adcrustations of callose on their walls (Ashford and Knox, unpublished data). This appears to be the only report of callose accumulation in stylar cell walls. Callose has been recorded in stylar cell walls following compatible pollinations in cotton (Jensen and Fisher 1969).

In interspecific and intergeneric matings in *Rhododendron,* pollen tubes showed a variety of responses, both in terms of tube and tube tip cytology, and their callose content (Williams et al. 1982, 1983). An interesting question is whether the callose response in one species of pollen varies following pollination on pistils of other species? In *Rhododendron,* striking differences have been observed in both the appearance of the tubes, and the number, shape and size of callose plugs, and these changes proved to be reproducible and predictable (Williams et al. 1983).

From these observations we can conclude:

– the callose response provides evidence for a dialogue between pollen grains and the stigma, with taxonomic distance of the pollen species providing defined limits for recognition.

– the phenotype of the response is similar to that of self-incompatibility, suggesting control through the expression of the *S*-gene or its products.

Pollen elicitor for the stigma callose response

Various pollen wall-associated fractions have been shown to elicit the stigma callose response: in *Brassica* and *Iberis,* pollen diffusates and proteins, MW range 10–25K (Heslop-Harrison et al. 1973, 1974, 1975); in *Raphanus;* pollencoat substances (Dickinson and Lewis 1973 b) and proteins (Dickinson and Lewis 1975). These proteins belong to the diploid sporophytic fraction (Heslop-Harrison 1975 b). Thus, the pollen elicitor is likely to be parentally-specified, like the *S*-gene, and its products found in the sporophytic self-incompatibility system. It remains to be isolated and characterized (Dumas and Gaude 1983 a).

In recent experiments exploring the specificity of the callose response, Sood et al. (1982) confirmed the response to self pollen at various stages of development. They also found that macerates of the anther primordia and other somatic tissues could induce a variable and weak callose response. These authors used macerates of fresh tissues for much of their work rather than diffusates although macerates are likely to contain elicitors for the wound programme.

The stigmas were not fixed before the ABF technique, a procedure also likely to induce the wound programme. Somatic tissue diffusates were employed by Kerhoas et al. (1983), of known protein content, and these failed to elicit a callose response. It seems likely that the low scores recorded by Sood et al. (1982) represent the 'noise' in their system; furthermore, the validity of their 'quantitative' differences between preparations is questionable, since the ABF method is at present entirely qualitative.

Cell surface probes have recently been employed to examine the specificity of the callose response in *Brassica* stigmas. Firstly, removal of the stigma surface pellicle by protease digestion prevented self pollen from inducing the callose response (Shivanna et al. 1978). Secondly the response to self or *Sinapis* pollen can be blocked by pretreatment of stigmas with the lectin concanavalin A (Kerhoas et al. 1983). This lectin binds to, and perhaps masks, the pellicle, the stigma surface layer (Dumas and Gaude 1981). Pre-treatment with the detergent Triton X-100 is almost as effective in blocking the response. This detergent is known to solubilize or alter the properties of membranes (Helenius and Simons 1975), and has recently been shown to prevent adhesion of *Brassica* pollen to stigmas (Singh et al. 1983). The response of unpollinated stigmas to self pollen diffusates is also blocked by both of these pretreatments (Kerhoas et al. 1983). Only a small amount of elicitor is required in the diffusate; 3 μg protein content was sufficient to induce a visible response within 2 min of application.

It is likely that receptors on the stigma surface pellicle interact with the primary signal to induce callose synthesis. This view is supported by results of Nishio and Hinata (1980, 1982) who, using an electrophoretic method, have shown that the *S*-specific glycoprotein of *Brassica* stigmas binds to Con A. It is also likely that other physical and chemical treatments known to overcome the incompatibility response, may have similar effects on the callose response: e.g. high levels of atmospheric CO_2 block the response in *Brassica* (M. B. Singh, personal communication 1983).

From these observations, we may conclude:

– recognition and receipt of pollen signals occurs at the stigma surface pellicle since binding, masking or solubilizing receptors blocks the response. Control thus appears to be exerted by sophisticated surface receptors.
– callose synthesis in the stigma always accompanies rejection and the same elicitor may trigger both responses.
– treatments which block rejection (absence of callose response) probably also block the incompatibility reaction.

Rapid determination of callose for plant breeding

We now give a brief review of the protocols available for the ABF method. It is desirable that the pistils be fixed rapidly – this minimizes any wound callose response. For breeding purposes, several authors claim it is sufficient to clear and stain tissue directly: e.g. *Prunus* (Jefferies and Belcher 1974) or *Corylus* (Thompson 1977; Me and Radicati 1983). Sometimes, as in the case of *Corylus,* this is absolutely essential as the aim has been to observe the number and extent of any pollen germination and tube growth on the stigma surface; after fixation, all grains are washed off and lost. However, the experiences of Hughes and Gunning (1980), who observed artefactual callose deposition throughout their tissue following aldehyde fixation, should be borne in mind, and where possible a rapid fixation schedule should be included in the experimental design.

Fixation may be readily and rapidly achieved by classical methods: e.g. with ethanol-acetic acid (3 : 1, V/V for 1 h at room temperature followed by storage in 70% ethanol); or by FAA (formalin : acetic acid : 70% ethanol in the proportions 5 : 5 : 90 V/V respectively, fix for 1 h or more at room temperature, material may be stored in fixative, which must be prepared fresh before use).

After fixation, the pistils are generally cleared to remove components that may quench the specific ABF reaction in the pollen tubes, and to facilitate squash preparation. Two methods are widely used:

– autoclave tissue in 50 g/l sodium sulfite for 10 min to 1 h at 121 °C; rapid and minimal damage to tissue (Jefferies and Belcher 1974).
– treat pistils with 8N or saturated solution of NaOH for 8 h or longer at room temperature (Martin 1959).

Aniline blue is a triarylmethane dye containing several minor impurities, among which is the fluorochrome which complexes with callose and other cell wall polysaccharides (Eschrich and Currier 1964). The fluorochrome has been isolated (review by Smith and McCully 1978), and recently purified, characterized and chemically synthesized (Evans and Hoyne 1982 and Evans, personal communication 1983). It contains two para-substituted aromatic rings with the formula $C_{25}H_{18}N_2Na_2O_7$, H_2O (Fig. 3).

There are today three methods for the ABF technique:

– the method of Currier (1957) and Linskens and Esser (1957): pistils are stained with 0.005 or 0.01% aniline blue prepared in 0.15 M K_2HPO_4 (pH approx. 8.2). This method is best for semithin sections and could also be useful in vital staining.
– the method of Martin (1959): stain pistils in 0.05 or 0.1% aniline blue prepared in 0.1 M K_3PO_4 (note pH approx. 12.4). This method gives a high intensity of fluorescence.
– use of fluorochrome. When this material becomes commercially available, an advantage will be its increased specificity of staining and the possibility to check quantitation of fluorescence. It binds to callose at a wide range of pH, so may readily be used with counterstains.

With the use of aniline blue, it is desirable to select a stain that will decolorize readily, so as to avoid the problem of the dye staining tissue components which then quench the specific fluorescence. Good results have been obtained with Merck Anilinblau WS (China Blue) or water-soluble aniline blue from other suppliers, including Fisher Scientific Co (CI no 42755). After decolorizing, stain should be made up with 10 or 20% glycerol to prevent squash preparations from drying out.

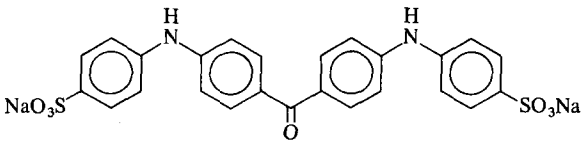

Fig. 3. Structure of the fluorochrome that binds to callose in the ABF method (Evans and Hoyne 1982)

Several counterstains have been employed with aniline blue: 0.7% calcofluor white to increase fluorescence of tubes (Jefferies and Belcher 1974), 0.01% acridine orange in 0.01 M phosphate buffer, pH 7.5 or 8.5 for 10 min to visualize cytological features (Alves et al. 1968), and 0.01% ethidium bromide in phosphate buffer to enhance fluorescence of pollen grains attached to stigma.

Role of callose

Callose, in appearance, is uncoloured and gelatinous, amorphous and isotropic (Herth et al. 1974), and is characterized by its solubility properties:

– insoluble in water but the presence of 1,4 β-linkages in a mixed link glucan may increase its solubility (Clarke and Stone 1963). Callose also has a remarkable water binding capacity – 84% of its dry weight may be lost on lyophilization (Vithanage et al. 1980). These properties are altered by calcium ions (Eschrich 1975).
– soluble in concentrated sulfuric acid, dilute KOH, calcium and stannous chlorides (Preston 1979).
– soluble in alcohol, but depends on degree of polymerization (Herth et al. 1974).

The chemical nature of callose has been the subject of considerable controversy, and is considered in detail by Clarke and Stone (1983). Here, it is sufficient to note the data relevant to pollination. Callose produced in pollen tubes of rye after self pollination proved to comprise a mixture of 1,3 and 1,4 β-linked glucans in the proportion of 9 : 77 (Vithanage et al. 1980). Reynolds and Dashek (1976) found that lily pollen tube callose stained with the ABF method, but not following protease treatment, suggesting the callose may be a glycoprotein. Dickinson and Lewis (1973 a) could not detect any protein-staining of callose in stigmas of *Raphanus*.

There has been considerable speculation on the possible role of callose (Dumas and Gaude 1983 b) since it is so strategically sited at the pollen-stigma interface:

– that it prevents tissue dehydration through control of cell water equilibrium by the intervention of calcium and potassium ions. Calcium ions block water molecules on the surface of callose; potassium ions liberate these water molecules (Eschrich 1975; Vithanage et al. 1980).
– it mobilizes reserve carbohydrate, according to the transitory nature of callose deposits (Currier 1957).
– that it takes part in defence reactions. Callose plays both an active and passive role in incompatibility; is related to stress responses, both trauma and environment (Vithanage and Knox 1977; Aist 1976; Lewis

1980), by isolating or sealing pollen from the stigma (Heslop-Harrison 1975 a; de Nettancourt 1977).
– it has a trophic role. Callose formation utilizes substrate that would otherwise be available for tube growth (Sedgley 1977).
– a physiological role in pollen tube growth: in vitro growth activated by 1,3 β-glucanases (Reynolds and Dashek 1976). These enzymes may act during growth in vivo to maintain tip growth through control of balance of wall-synthesizing and degrading enzymes, as suggested for fungal hyphae (Bartnicki-Garcia 1973). Callose accumulation in incompatible tubes could be due to a change in balance.

A model based on the parallels between pollen-pistil and host-pathogen interactions is given in Fig. 4. Linskens (1975, 1976, 1980) proposed that operationally the stigma may be programmed to reject all pollen tubes, which would be regarded as pathogens or parasites, according to the physiological concepts of heterotrophic nutrition (Loewus and Labarca 1973). For compatible pollen, the difference would reside in the destruction of the rejection mechanism through the action of a pool of enzymes following recognition at the stigma surface. This means that the callose formed would be continuously degraded (Linskens 1975). In Fig. 4, callose formation is preprogrammed in both

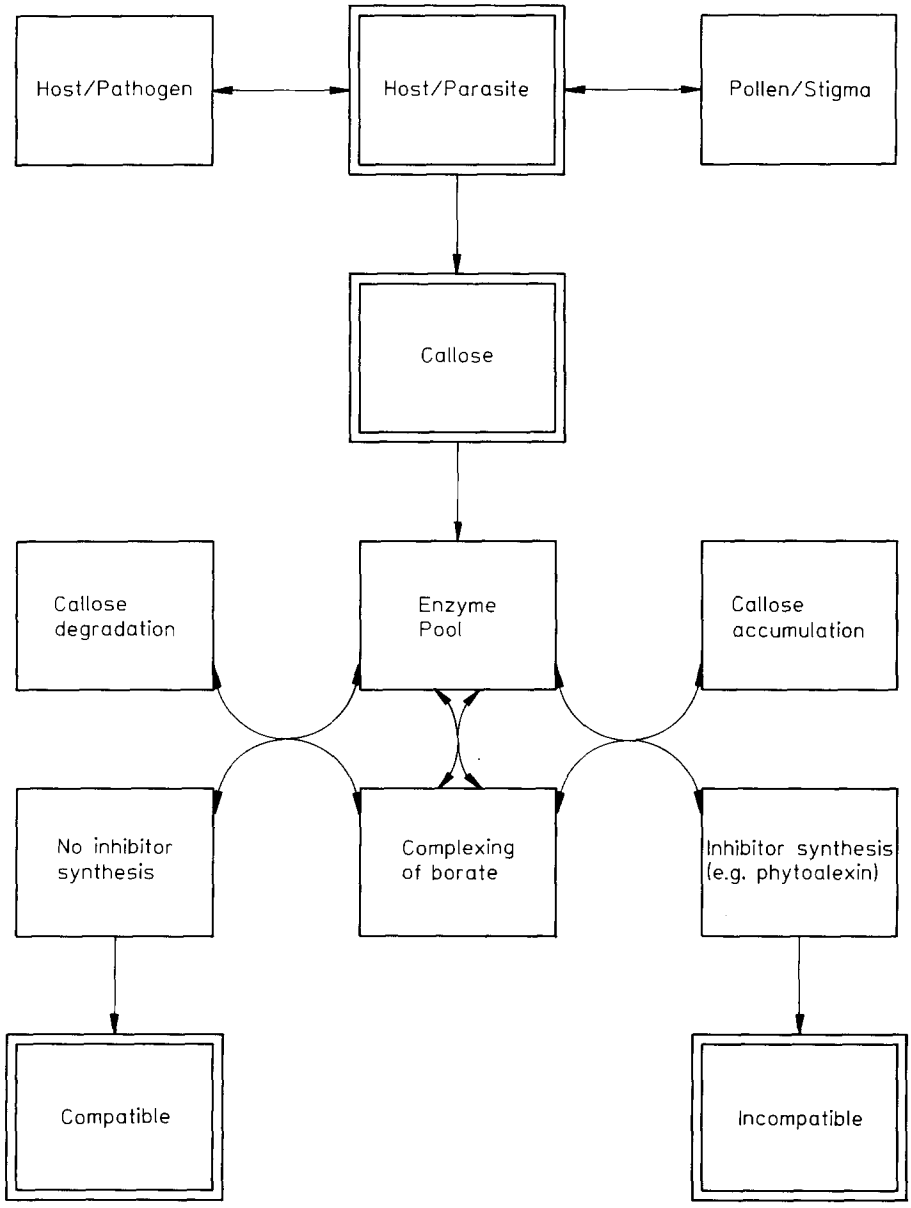

Fig. 4. Summary scheme showing current hypotheses concerning callose synthesis, degradation by hydrolytic enzymes (Linskens 1976; Stösser and Hohl 1981), borate-complexing and ihibitor production (e.g. phytoalexin-like compound) in self-incompatible pollination (Lewis 1980), as described in text

compatible and incompatible pollinations, and degradation during compatible interactions is shown in Pathway 1. In incompatible interactions (Pathway 2), callose accumulates as the enzyme pool is inhibited. The model also incorporates the hypothesis of Lewis (1980) on the utilization of boron. In the incompatible interaction, borate may be sequestered by callose, producing a borate deficiency, and altering polyphenol metabolism so that phytoalexin-like substances are produced (Pathway 2, Fig. 4). These are inhibitors of microbial pathogens, including pollen as shown by the inhibitory effect of rishitin on pollen tube growth in vitro (Hodgkin and Lyon 1979).

Resistant host plants secrete larger amounts of phytoalexins than susceptible plants, and it is likely that these compounds could be involved in incompatibility responses in the stigma since they inhibit endo- and exo-glucanases (Stösser and Hohl 1981). Such enzymes are also found in the male gametophyte (Reynolds and Dashek 1976). Borate is known to be necessary for pollen tube growth (Stanley and Linskens 1974). In the compatible situation, there is no shortage of boron as callose is continuously degraded.

Future prospects

Callose, as detected by the ABF method, provides a useful bioassay in two situations:

- pistil receptivity and viability
- pistil incompatibility.

There is an urgent need for the callose response to become a quantitative test, and to make progress in understanding the genetic control of callose synthesis. The fact that this metabolic response occurs in such a wide range of situations in flowering plants suggests that plants have a unique response. This may be induced by a factor with a common region in its molecule and parts specific for the different functions related to recognition: e.g. microbial pathogens, stigma or pollen (Dumas and Gaude 1982). A range of proteins and glycoproteins exist at these interfaces, e.g. pollen and stigma surfaces (Knox 1983). To date, only *S*-specific glycoproteins of pistils have been characterised (Ferrari et al. 1981; Mau et al. 1982; Nishio and Hinata 1982). These glycoproteins may function in reading-out information e.g. *S*-gene specificity, from the pollen. Little is known of the interaction, and it may be regarded as a black box phenomenon. To modify the read-out or information received from the pollen or transmitted by the stigma is the aim of plant breeders when attempting to create new hybrid crops. This is today a neglected part of the biotechnology of seed production. The callose response provides a useful tool with which to explore the reproductive processes involved.

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