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# THE POLLEN-STIGMA INTERACTION: BUD POLLINATION IN THE CRUCIFERAE

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SUMMARY

The stigmas of buds of the age range capable of accepting self-pollen (bud pollination) in self incompatible individuals of *Raphanus sativus* and *Cheiranthus cheiri* were found to have cuticularised papillae already carrying a thin surface secretion over the receptive area. Enzymatic digestion of the proteinaceous part of this secretion reduced the germination rate of both compatible and incompatible pollen and prevented the tubes that were formed from penetrating the cuticle of the papillae, so abolishing the self compatibility. This indicates that in buds of an age capable of accepting self-pollen the stigma surface factors concerned with the self-incompatibility response have not yet accumulated in quantities sufficient to inhibit pollen germination and tube growth, although those involved in promoting germination and facilitating the entry of the tubes are already present. The behaviour of the Cruciferae which do not accept self-pollination in buds of any age, such as a variety of *Sinapis alba* tested, may be explained on the assumption that the stigma surface factors concerned in germination and tube penetration are secreted simultaneously with the incompatibility factors so that there is no period when the incompatible pollen and pollen tubes are both free from the inhibitions imposed by the self-incompatibility system and capable of penetrating the stigma.

## 1. INTRODUCTION

In the homomorphic sporophytic self incompatibility (SI) system of the Cruciferae, pollen derived from a parent of an incompatible genotype is inhibited on the stigma surface or shortly after the penetration of the tube tip into a surface papilla (STOUT 1931; KROH 1964; LINSKENS & KROH 1967). The inhibition of the pollen is usually followed by the accumulation in the grain or tube of the $\beta$-1,3-glucan, callose, and a counterpart reaction, the deposition of a lenticule of callose at the plasmalemma, generally – but not invariably – develops in stigma papillae that are in close contact with incompatible grains (HESLOP-HARRISON, HESLOP-HARRISON & KNOX 1973). The fact that the control of the incompatibility response on the pollen side is by the genome of the pollen *parent* (BATEMAN 1955) led to the suggestion that the *S*-gene products concerned are derived from the tapetum, a diploid parental tissue, and conveyed to the stigma in the pollen wall (J. HESLOP-HARRISON 1968). Proteins derived from the exines of pollen grains from incompatible plants can indeed induce callose formation in stigma papillae, providing some support for this view

(DICKINSON & LEWIS 1973b; HESLOP-HARRISON, KNOX & HESLOP-HARRISON 1974).

Materials held in the exine pass out onto the stigma surface after pollination, and are received by an extracuticular proteinaceous film or pellicle which coats the receptive part of the mature stigma papillae (MATTSSON et al. 1974). This coating contributes to the antigens present in extracts and diffusates of crucifers such as the cultivars of *Brassica oleracea* (HESLOP-HARRISON, HESLOP-HARRISON & BARBER 1975). Such extracts contain antigens specific for the S-alleles present in the stigma (NASRALLAH, BARBER & WALLACE 1970), and the fact that these are lost readily from intact stigmas suggests that they are held on or in the surface papillae. Furthermore, FERRARI & WALLACE (1975) have reported that diffusates of intact stigmas inhibit the germination and growth of self (incompatible) pollen *in vitro*, while not affecting germination and tube growth of pollen from parents of compatible genotypes. Taken together, these results suggest that the recognition reaction of the SI system takes place on the stigma surface, involving, on the one hand, the exine-borne proteins from the pollen, and, on the other, factors carried on or near to the surfaces of the receptive parts of the stigma papillae (J. HESLOP-HARRISON, 1975a, 1975b, 1975c & 1975d).

The investigation of pollen-stigma relationships in the Cruciferae is complicated by the circumstances that, simultaneously with the operation of the SI system, other interactions are taking place which can effect the success or failure of pollination. In this family as in many others the emerging tube tip must penetrate the cuticle of a contiguous stigma papilla before the passage through the style can begin, and this apparently requires the activation of cutin-esterase ("cutinase") at the point of entry (LINSKENS & HEINEN 1962). Impressed by the effectiveness of the control that the cuticle could perhaps provide, CHRIST (1959) suggested that the SI system in the Cruciferae might be related to the capacity of the stigma to activate a pollen-borne cutinase precursor. This hypothesis can hardly now be sustained for the intraspecific SI system (KANNO & HINATA 1969), but it is conceivable that maladjustments between stigma and pollen affecting the capacity of the tube to enter could act as interspecific incompatibility barriers (J. HESLOP-HARRISON 1976). For convenience of reference, we have designated the control over stigma penetration as System 1, and under this same heading could be grouped other forms of stigma-pollen complementation the failure of which could lead to the prevention of fertilisation. System 2 then comprises the recognition event and subsequent responses in which S-allele specificity is expressed (J. HESLOP-HARRISON 1975b).

The fact that several interactions occur on the stigma during the first few minutes after pollination confuses the interpretation of experiments where the presumed surface receptor sites are blocked or disrupted. In self-incompatible crucifers, pollen germination is not wholly prevented when the surface proteins of the stigma are removed enzymically, but the tubes often fail to penetrate in both compatible and incompatible pollinations (J. HESLOP-HARRISON 1975a). However, the same effect is seen in other species without SI

systems (HESLOP-HARRISON & HESLOP-HARRISON 1975; Y. HESLOP-HARRISON 1977). Evidently, then, although the removal of the receptor sites may impinge on the working of System 2, any such effect cannot readily be disentangled from that on System 1.

There is a similar difficulty in understanding the rationale of bud pollination. In many SI crucifers, immature buds of a certain age class will accept incompatible pollen, and the fact has long been exploited as a method of defeating the SI system in crop breeding. Its success in such genera as *Brassica* and *Raphanus* certainly suggests that the stigma factors inhibiting the pollen in incompatible combinations are not yet present or are inactive in buds of an age accepting self-pollen, and indicates, furthermore, that no other barriers are present in such buds. Among these barriers could be the cuticle of the papillae, which in a mature stigma must be disrupted before tube entry is possible. Is the cuticle not a significant barrier in buds of an age where self-pollination is possible? Or is the presumed cutinase activator already present on the stigma surface in such buds? The possibilities might be distinguishable as follows:
(a) If the cuticle is not yet an effective barrier in buds showing self compatibility, this should be apparent from the structure of the walls of the stigma papillae, and if in such buds the stigma receptors are absent, this might be detectable from the properties of the papilla surface.
(b) If the cuticle is an effective barrier so that the pollen-borne cutinase precursor must be activated before it can be penetrated (System 1), the implication would be that the activator is secreted *before* the factors concerned in the SI response (System 2). Removal of the surface materials should then make it impossible for the tubes to penetrate the stigma even in buds of the age class permitting self-pollination, so abolishing the self incompatibility.

In this paper we report the result of experiments designed to investigate these possibilities.

## 2. MATERIALS AND METHODS

The experiments were carried out with *Raphanus sativus* L. (cultivars including "Iceberg", Suttons & Sons, Reading), *Cheiranthus cheiri* L. (Royal Botanic Gardens, Kew, accessions 006/74 00210 and 00211, wild source), and *Sinapis alba* L. (RBG, Kew stock, source unknown), The samples of *R. sativus* and *S. alba* were grown in open plots during the spring and summer of 1974 and 1975 and the inflorescences of *C. cheiri* used were taken from plants grown under glass and in the open.

It is almost always found that the effectiveness of the SI system of the crucifers varies according to plant age, and the control is often sensitive to temperature and other environmental conditions during the flowering period. The stocks used in the present work showed much variation, and most of the comparisons were therefore made with selected plants in active growth which were tested beforehand to establish that they showed a satisfactorily high degree of

self-pollen rejection. Because of the variation often apparent between flowering axes of different age both in developmental rate and effectiveness of the SI response, the buds used were taken from matched inflorescences. These were brought into the laboratory usually the day before the tests were made, and retained in diffuse light at a temperature of 20–24 °C.

The controlled pollinations were made using matched buds with perianth removed or opened out to expose the pistil, implanted in 1% agar in petri dishes. Pollen was taken from freshly dehisced anthers, and its viability estimated from germination rate on control stigmas or fluorochromatically (HESLOP-HARRISON & HESLOP-HARRISON 1970). The pollinations were carried out with a fine brush. The operation was performed under a binocular dissecting microscope, and the pollen was carefully distributed over the stigma surface to ensure that the highest possible proportion of the grains applied came into direct contact with stigma papillae.

Dishes with pollinated stigmas were kept at room temperature (23–24 °C) for 3–5 h in diffuse light before mounting for observation. Normal pollen tubes of the Cruciferae can readily be localised on the stigma and in the style using the method of LINSKENS & ESSER (1957) which depends on the detection of the callosic inner lining of the tube by its fluorescence following staining with decolorised aniline blue. The callose deposits frequently developed in inhibited tubes and adjacent stigma papillae after incompatible pollination can be detected with the same staining procedure. The staining was carried out with aniline blue (BDH) in a concentration of 0.005% in phosphate buffer at pH 9.5, and observations were made with a Vickers Photoplan system using epi-illumination and epi-objectives with Vickers exciter filter No. 1 and barrier filter No. 3. The pollinated stigmas were either transferred directly to the stain for observation, or where necessary fixed in acetic-alcohol (1:3, v/v) followed by 70% alcohol for storage before staining. Every precaution was taken to avoid loss of pollen from the stigma surface during mounting. This is particularly important in incompatible pollinations where the pollen does not become attached firmly to the stigma surface when there is no tube penetration. Small losses of pollen probably did occur in these cases, with the effect of reducing slightly the proportions of grains recorded as failing to produce a tube.

In general, not less than 100 grains were recorded for each pollination. A grain was scored as "germinated" if the tube tip had emerged and extended as least half the diameter of the grain. A tube was recorded as having entered the stigma if the tip had passed through the cuticle of a papilla and made contact with the underlying pectocellulosic wall. The proportion of pollen grains or tubes associated with the callose rejection reactions in adjacent stigma papillae was scored for each pollination, and the type of reaction – whether strongly localised or diffuse – was recorded.

The appearance of esterase activity on the surface of the stigma papillae provides an indication of the onset of secretion (MATTSSON et al. 1974). The method of PEARSE (1972) was used to localise esterase activity, using α-naphthyl acetate as a substrate in a coupling reaction with Fast Blue B salt. Cutin was

detected with the fluorescent stain, auramine-O (Y. HESLOP-HARRISON 1977), made up at 0.01% in 0.05 M tris-HCl buffer, pH 7.2. Fresh or fixed material was mounted in the stain solution for direct observation, or transferred to dilute glycerine after staining to saturation.

To follow the effects of dispersing the secreted proteins of the stigma papillae, stigmas were incubated for periods of 30–40 min in Pronase (BDH, 1 mg per ml in 0.05 M tris-HCl buffer at pH 7.2 and 8% sucrose) at room temperature, a treatment known to remove esterase activity and the capacity for binding Concanavalin A from stigmas bearing surface protein films (HESLOP-HARRISON, HESLOP-HARRISON & BARBER 1975; KNOX et al. 1976). The stigmas were left attached to the pistils, which were inverted in the enzyme solution so that the receptive heads were immersed with the cut faces above the meniscus. To ensure close contact with the medium and to remove trapped air bubbles the immersed stigmas were put under reduced pressure for a few minutes, after which any remaining air bubbles were carefully brushed away. Following the digestion period the stigmas were rinsed once more with buffered sucrose, followed by distilled water for two or three minutes, drained onto filter paper, and implanted in agar for pollination. Controls were treated for the same periods in buffered sucrose alone, with the same washings.

Pollinated and unpollinated stigmas for freeze-sectioning were encased in 15% gelatine and sectioned on a SLEE cryostat at −15 °C, without prior fixation. Material for wax-embedding and sectioning was fixed in 3% glutaraldehyde buffered at pH 7.0 in 0.05 M phosphate buffer with 8% sucrose, usually overnight, before dehydration through an alcohol series.

## 3. PRELIMINARY OBSERVATIONS

The effectiveness of the SI system as estimated by the rejection of self pollen varied to some extent between individuals of *R. sativus* and *C. cheiri*, but even in those showing the most positive inhibition of self pollen a generation of buds could be identified which showed self compatibility judged both by the germination of self pollen and the subsequent penetration of pollen tubes, and by seed set following selfing. This generation of buds could be specified by reference to bud length, measured from the point of insertion of the calyx members to their tips, provided that comparisons were made between inflorescences of the same order. For the tests *in vitro*, buds from matched inflorescences were therefore grouped into size classes chosen to cover the age range showing self compatibility and later developmental stages in which self compatibility had been lost. With *R. sativus*, tests were also carried out with a range of buds younger than those showing self compatibility.

The race of *S. alba* tested showed no capacity for bud-pollination, and it therefore provided a useful comparison with *R. sativus* and *C. cheiri*. Buds of corresponding size ranges relative to the open flower were selected for the pollinations *in vitro*.

## 4.1. Raphanus sativus

### 4.1.1. Bud Pollination

The outcome of bud pollination using three highly self incompatible plants of *R. sativus* is recorded in *fig. 1A*. Only in buds of the size range 4.0–5.0 mm did the stigma accept self pollen; in these rather more than 25% of the tubes entered the stigma.

The proportions of stigma papillae producing callose in response to contact with incompatible pollen grains is shown in *fig. 1B*. Only in the oldest buds and flowers was a localised callose response found, when about 40% of the papillae reacted. In the class of buds showing self compatibility most of the stigma papillae showing any reaction at all produced diffuse callose and the proportion with this response was greatest in buds of the youngest class.

The contrast between diffuse and localised callose responses may be seen in *figs. 2A–D*. In general, papillae in mature flowers showing the rejection response produce lenticular callose deposits on the inner surface of the papilla wall. The structure of these in *R. sativus* has been described in the electron microscopic study of DICKINSON & LEWIS (1973a), who showed that the callose is laid down at the plasmalemma opposite the contact face with the adherent pollen grain. This type of reaction is seen in most of the papillae of the stigma fragment illustrated in *fig. 2A*. Buds of an age class showing self compatibility produced some localised callose, but this was generally accompanied by the formation of dispersed granules elsewhere in association with the plasmalemma of the receptive part of the papilla. In buds of the next youngest size range, ring-shaped deposits were occasionally found (*fig. 2B*), but mostly the response where apparent at all was diffuse, the deposit extending over the plasmalem-

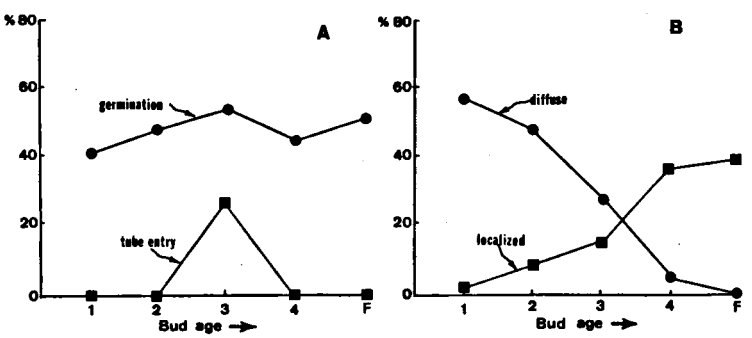


Fig. 1. *Raphanus sativus*. Response of stigmas from developing buds and the freshly-opened flower to incompatible pollination. A, percentages of pollen-grain germination and pollen-tube entry into the stigma papillae. B, percentages of papillae associated with incompatible pollen grains forming localised callose deposits (cf. *fig. 2A*), or diffuse or annular deposits (cf. *fig. 2B–D*). Pooled data of three experiments, three stigmas per treatment in each. Bud length (age) classes, 1, 2.0–2.5 mm; 2, 3.0–3.5 mm; 3, 4.0–5.0 mm; 4, 7.0–8.0 mm; F, freshly opened flowers. Scored 5 h after pollination.

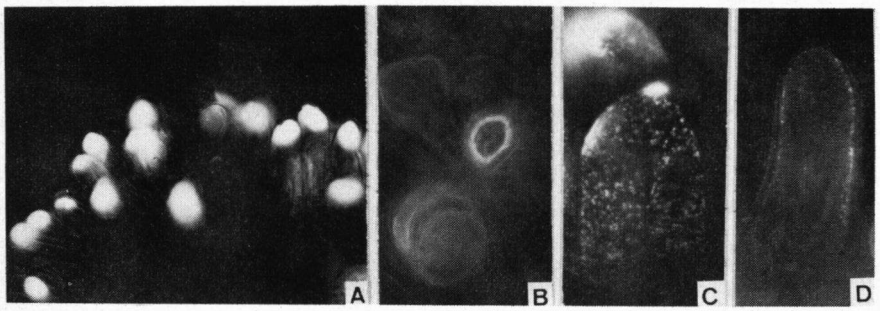


Fig. 2. *Raphanus sativus*. Types of callose reaction produced in papillae 5 h after pollination with incompatible pollen grains. Fluorescence micrographs following staining with decolorised aniline blue. A, portion of a mature stigma head showing the typical reaction, with a lenticule of callose formed at the plasmalemma of each papilla that has been in contact with an incompatible grain. × c. 270 . B, annular callose deposit formed in a papilla from a bud, typical of those produced by stigmas of the age ranges 1–3 of *fig. 1* after contact with incompatible pollen grains. Such rings may be formed with or without associated dispersed deposits like those of C and D. × c. 750. C, diffuse callose reaction, with dispersed granules of callose giving a spangled appearance. × c. 1100. D, as in C, but focused so as to show the association of the callose with the plasmalemma. × c. 800.

ma, either as a thin film, or in dispersed granules giving a spangled appearance (*fig. 2C, D*).

4.1.2. Developmental state of the stigma in buds showing self compatibility

In *R. sativus* the extension of the epidermal cells of the receptive face of the stigma begins in buds of the size range 1.5–2.0 mm. From this early time a cuticle is present, and although it may thin out for a period during the subsequent rapid elongation of the papillae, there is evidently a continued secretion of cutin precursors into the wall so that the layer is never wholly disrupted. In buds of the size range 4.0–5.0 mm – that is, those susceptible to self pollination – the papillae are well formed and already extended to three-quarters of their mature length. DICKINSON & LEWIS (1973a) have provided a description of the ultrastructure of the papillae of *R. sativus* from buds of a slightly later stage, illustrating in these a cuticle of about 30 nm in thickness. At the time of anthesis, the receptive tips of the papillae bear an irregular cutinised layer 30–40 nm thick, made up of short, radially oriented columns, a structure comparable with that found in other families with dry papillate stigmas (HESLOP-HARRISON et al. 1975).

The first surface esterase activity over the receptive part of the stigma papillae can be detected cytochemically in buds in the size range 3.0–5.0 mm; however, the main period of secretion is during the period of bud growth from 5 to 10 mm, continuing during anthesis (HESLOP-HARRISON, HESLOP-HARRISON & BARBER 1975).

The tips of the stigma papillae of buds of the size range showing self compat-

ibility have thus already (a) a thin but well-defined cuticle and (b) a slight, but cytochemically detectable, extracuticular surface secretion.

### 4.1.3. Effect of stripping the surface secretion layer of the stigma papillae

Enzymic disruption of the surface materials resulted in an appreciable decrease in pollen germination on the mature stigmas and on those from buds of the age class showing self-compatibility (*table 1*). At the same time, the capacity of the emergent tubes to enter the papillae in the potentially self compatible buds was abolished.

The removal of the surface secretion had some effect also on the localisation of the callose in those papillae revealing a rejection response (*table 1*). In mature stigmas, the proportion showing strongly localised responses comparable with that of *fig 2A* was lower, and most of the affected papillae showed a diffuse reaction, occasionally with annular callose deposits. In the potentially self compatible buds, the capacity to produce localised callose was much reduced, although one-quarter of the papillae continued to give a diffuse reaction.

Table 1. Effect of enzymic digestion of stigma surface materials on pollen germination, tube penetration and the formation of callose in stigma papillae following incompatible pollination of (a) flowers at anthesis and (b) potentially self compatible buds (size range 4.0–5.0 mm) of *Raphanus sativus*.

| | (a) Flowers at anthesis | | | (b) Potentially self-compatible buds | | |
|---|---|---|---|---|---|---|
| | Untreated control | Buffer washed control | Pronase digested | Untreated control | Buffer-washed control | Pronase digested |
| Germination | 57.14 | 56.86 | 31.25 | 61.42 | 58.00 | 31.35 |
| Tube entry | 0.00 | 0.00 | 0.00 | 25.00 | 16.00 | 0.00 |
| Localised callose | 40.47 | 35.29 | 18.75 | 10.00 | 10.00 | 1.25 |
| Diffuse or annular callose | 0.00 | 0.00 | 29.16 | 30.00 | 18.00 | 25.00 |

NOTE: Mean percentages from the pooled results of two experiments, three stigmas per treatment. "Localised" callose as in *fig. 2A*; "diffuse" or "annular" callose as in *figs. 2B–D*.

## 4.2. Cheiranthus cheiri

### 4.2.1. Bud pollination

The results of self- and cross-pollination in flowers at anthesis and buds of three size classes are given in *fig. 3*. In *C. cheiri*, the germination of self pollen was high on the younger stigmas, reaching the proportion shown by compatible pollen in buds of the size range 2.5–3.0 mm. The tubes entered the stigma moderately freely in buds of this size range, and more than one third of the tubes emerging penetrated even in the next largest size range.

As in the experiments with *R. sativus*, the numbers of stigma papillae in

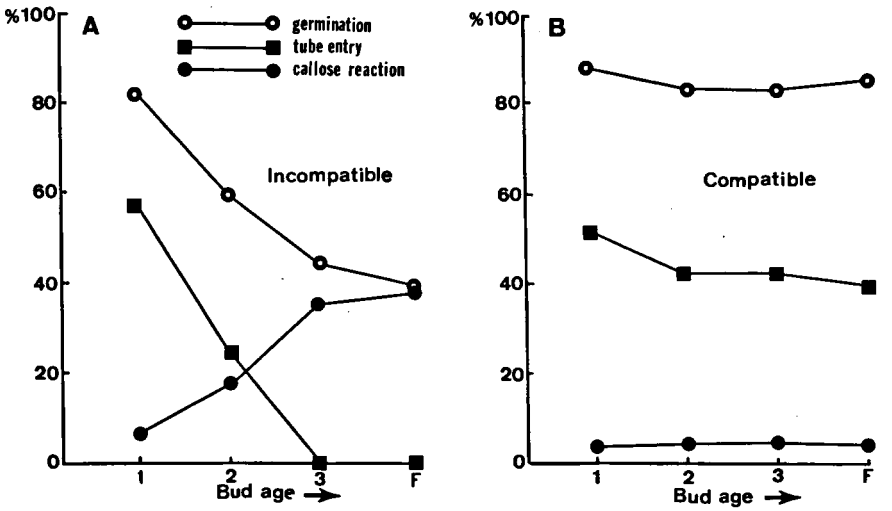


Fig. 3. *Cheiranthus cheiri.* Response to incompatible (A) and compatible (B) pollinations in developing buds and the freshly opened flower. Pooled results of two experiments, two stigmas per treatment in each. Bud length (age) classes 1, 2.5–3.0 mm (pistil, 1.5–2.0 mm); 2, 5.0–5.5 mm (pistil, 2.5–3.0 mm); 3, 7.0–8.0 mm (pistil, 4.0–4.5 mm); F, open flower. Scored 5 h after pollination.

contact with grains with localised rejection reactions were lower in buds of the age range capable of being selfed. In a few selfed papillae of pistels of the youngest age class where the tube had entered, small dispersed patches of callose were present at the plasmalemma. Diffuse callose deposits comparable with those seen in bud-pollinated *R. sativus* (*fig. 2C, D*) were not observed in *C. cheiri.*

### 4.2.2. Developmental state of the stigma in buds showing self compatibility

As in *R. sativus*, a cuticle is present on the papillae of *C. cheiri* from the time of their initiation. Older papillae show marked discontinuities over the receptive area, visible with the optical microscope after auramine-O staining. Slight surface esterase activity was detected in buds of the size range accepting selfing, suggesting that secretion had begun. The developmental state of the stigmas accepting self pollen in *C. cheiri* was therefore much as in self-compatible buds of *R. sativus* in respect both to cuticle and surface secretions.

## 4.3. Sinapis alba

Results of one experiment using a highly self incompatible plant are given in *fig. 4.* Buds at all the age ranges tested rejected self pollen, and the same situation was found in other plants of this population. The stigma papillae of the youngest buds tested showed less tendency to form localised callose deposits

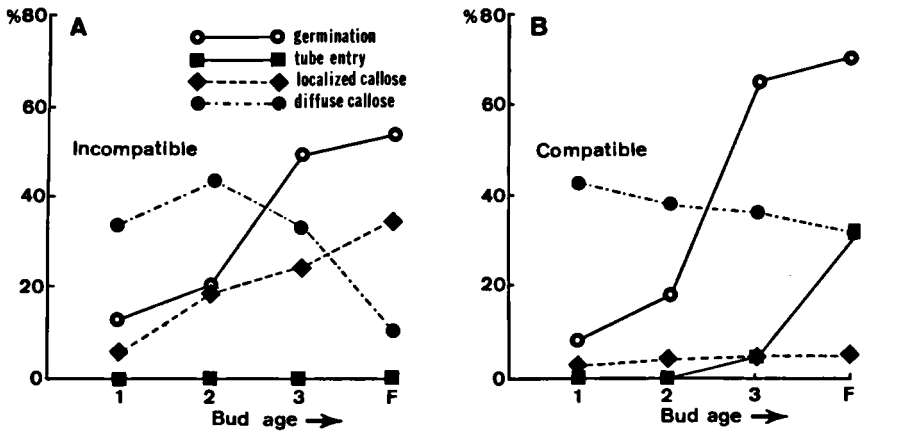


Fig. 4. *Sinapis alba.* Response to incompatible (A) compatible (B) pollinations in developing buds and the freshly opened flower. One experiment, two stigmas per treatment. Bud age classes comparable with those of *Cheiranthus cheiri, fig. 3.* In this species, diffuse callose deposits are associated with compatible pollinations quite frequently, and there is no period during which buds will accept incompatible pollen.

in association with adherent grains than did those at anthesis, but even in compatible pollination of mature stigmas some callose was commonly formed, often in diffuse or ring form.

## 5. DISCUSSION

*Raphanus sativus* and *Cheiranthus cheiri* both showed bud self compatibility in these experiments, and in each the stigmas of buds of the age range accepting self-pollen were found to have cuticularised papillae already carrying a thin surface secretion over the receptive area.

In *R. sativus* removal or disruption of the proteinaceous part of this secretion by enzymic digestion abolished the capacity of stigmas of this age range to accept self-pollen. This treatment also reduced the germination capacity of both compatible and incompatible pollen on these young stigmas, but it was clear from the behaviour of the tubes developed from those grains that did germinate that the ability to penetrate the cuticle of the stigma papillae had been lost. These results may be explained as follows.

(a) In a *compatible* pollination of a mature stigma, the pollen encounters surface materials which promote germination and activate enzyme systems concerned with the lysis of the cuticle and tube penetration. Such complementation effects would relate to System 1. No counteracting inhibitory effects arise from the function of System 2, since the combination is a compatible one.

(b) In an *incompatible* pollination, System 1 complementations proceed normally, but tubes are inhibited through the specific effect of System 2.

(c) In buds of an age accepting self pollen, the stigma surface factors concerned with the SI response (System 2) are not yet present in quantities sufficient to inhibit pollen germination and tube growth. In these buds, however, the factors involved in promoting germination and facilitating the entry of the tubes (System 1) are already present.

The behaviour of *Sinapis alba* and other Cruciferae which do not accept self-pollination in buds of any age can be explained on the assumption that there is some variation between species and genera in the timing of the various classes of secretion. In *S. alba*, even compatible pollen germinates poorly on the young stigmas, and compatible tubes are unable to penetrate the stigma surface except in open flowers and, minimally, in buds of the most mature class, so it must be concluded that the factors concerned in the complementations of System 1 appear later than they do in *Raphanus* and *Cheiranthus*. Assuming that the factors involved in the SI response in *S. alba* are secreted during the final maturation of the buds as in the other two species, it can be seen that no interval would exist during which bud pollination would be possible.

The above interpretation contains the assumption that the stigmas of the Cruciferae secrete factors which promote pollen germination, and that these overlie the receptive part of the stigma papillae, whence they are dispersed when the secretion is disrupted experimentally. This is the readiest explanation for the fact that pollen germination is reduced both in cross- and self-pollinations when the stigma surface materials are removed enzymatically. This conclusion seems not wholly compatible with the observations of FERRARI & WALLACE (1975), who have recently developed a medium in which the pollen of *Brassica* spp. readily germinates in the absence of stigma substances. Evidently their medium substitutes for whatever stigma-surface factors promote germination in the natural situation. It is not excluded, however, that the effect is a physical one related to the passage of water into the grain from the stigma papilla, and that the special property of the Ferrari-Wallace medium, which includes 20% polyethylene glycol, is its capacity to provide this regulation.

It is noteworthy that FERRARI & WALLACE (1975) report that stigma diffusates inhibit the germination of self-pollen in their medium, but not diffusates from stigmas of a different incompatibility genotype. The effective factors in these diffusates are presumably related to the SI response, forming part of System 2. In earlier work, NASRALLAH & WALLACE (1967) found that the *S*-gene specific antigens present in mature stigmas of *Brassica oleracea* were absent in younger buds, and suggested that this might explain the success of bud pollination in this genus. Our conclusions for *Raphanus* and *Cheiranthus* fully support the general interpretation offered by these authors, although direct evidence is still lacking to show that the S-gene specific antigens are indeed the factors involved in the System 2 recognitions.

Turning to the callose-deposition response in stigma papillae, it seems clear from the results recorded in this paper that this is related to the operation of the SI system, as various earlier studies have indicated. However, the timing of the response when it occurs shows that it is secondary, developing after the

primary recognition and the immediate consequent events; it cannot therefore be viewed as being directly concerned in the blockage of incompatible pollen. It is significant, however, that the deposition of callose following contact with incompatible grains is less in younger buds of *Raphanus* and *Cheiranthus*, and that when the papillae in the younger buds do react, it is often in a diffuse way, without the precise localisation opposite to the contiguous grain seen in the mature stigma. This incompetence could be due either (a) to the absence of localised System 2 receptor sites on the stigma surfaces or adjacent plasmalemmas in young buds, or (b) to the relative inactivity of the callose synthetase system in the plasmalemmas of the young papillae (HESLOP-HARRISON et al. 1975). Since the removal of the surface materials by enzymic digestion from mature stigmas of *Raphanus* also changes the form of callose deposits in incompatible pollinations, increasing at the same time the proportion of diffuse reactions, one may now conclude that explanation (a) is the more probable.

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