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Analysis of Stylar Self-Incompatibility Competence by Use of Heat Induced Inactivation

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Summary. Immersion of *Lilium longiflorum* pistils in 49° C water for increasing durations of 1,2,3, or 4 minutes immediately prior to incompatible pollination resulted in a correspondingly progressive decrease in the stylar self-incompatibility competence, as determined from the lengths attained by pollen tubes during 48 hours growth in the styles at 24° C. Neither pistils remaining on the plant nor those detached from the plant which were immersed after anthesis in 49° C water for 5 minutes regained self-incompatibility competence during a 48 hour incubation at 24° C prior to incompatible pollination. Heat treatment of detached pistils as early as 39 hours prior to bud anthesis also resulted in an inactivation of stylar self-incompatibility competence when incompatible pollination was made at 24 hours after anthesis. Experiments utilizing heat treatment of partial lengths of detached whole styles revealed that pollen tubes which have grown through as much at 45 millimeters of either a physiologically incompatible or compatible portion of the style are still capable of shifting to either a higher growth rate or lower growth rate upon entry into respectively either a physiologically compatible or incompatible portion of the style.

Introduction

Self-incompatibility in flowering plants is a genetically controlled system resulting in the inability of a plant producing functional gametes to set seed when self-pollinated. Identical specificities in pollen and pistil dictate this failure to set seed, and the genes coding these specificities are commonly referred to as S-alleles (self-incompatibility alleles). The observable physiological and cytological manifestation of the self-incompatibility interaction between pollen and pistil may occur on the stigma surface, within the style, or in the ovule prior to or following fertilization (Sears 1937; Bateman 1954; Brewbaker 1957; Rowlands 1964). The site of manifestation is characteristic of a species and tends to be the same for all self-incompatible species within a family (Brewbaker 1957; Pandey 1960).

In *Lilium longiflorum* the self-incompatibility reaction appears to be expressed in the upper third of the style; not at the stigmatic surface (Myodo 1962; Yamada 1965; Ascher 1967). Pollen tubes incompatible with the stylar tissue grow at much reduced rates compared to compatible pollen tubes.

Heat-induced inactivation of self-incompatibility in this species has recently been demonstrated. Pollen tubes growing in incompatible styles incubated at 39° C exhibit a growth rate nearly as high as that of pollen tubes growing in compatible styles at 39° C during a 48 hour period (Ascher and Peloquin 1966a). The site of this high temperature effect appears to be the style since a short, pre-anthesis immersion of the style in 50° C water for 6 minutes is equally effective in inactivating the self-incompatibility response (Hopper, Ascher and Peloquin 1967). The grow .h rate of incompatible pollen tubes at 24° C in the heat treated styles is similar to that of compatible pollen tubes growing at 24° C in non-pretreated styles when measured over a 48 hour period.

Temperature sensitivity of self-incompatibility has been found also in other species. Previous to the work with *Lilium longiflorum*, partial inactivation of self-incompatibility by heat had been demonstrated in *Oenothera rhombipetala* (Bali 1963) and in *Oenothera organensis* (Hecht 1964). For *O. organensis*, a 5-minute immersion of the pistil in 50° C water inactivated the self-incompatibility, whereas for *O. rhombipetala*, a 1 to $2\frac{1}{2}$-minute immersion was sufficient. These authors have interpreted their results as reflecting a heat-induced degradation of incompatibility substances in the stigma and style. Temperature sensitivity of self-incompatibility has been demonstrated also within the species *Trifolium hybridum*. One clone of tetraploid *T. hybridum* was found to change from incompatible to compatible after one to two days at constant 32° C or 32° C day and 27° C night temperature, and to revert to incompatible after 24 hours at constant 21° C (Townsend 1966). This temperature sensitivity was found to be due to a single dominant gene not associated with the S-locus genotype. Similar results were observed with

two diploid clones of *Trifolium hybridum* L., and for one of these clones the site of the change in the incompatibility was demonstrated to be the style (Townsend 1968). Townsend has suggested from these results that temperature may be acting as a switch in "turning on and off" the synthesis of an enzyme controlling the production of incompatibility substances in the style.

Results are reported here which further characterize the heat-induced inactivation of self-incompatibility in *Lilium longiflorum*. The progress of the heat-induced inactivation with respect to treatment duration was determined. Experiments were also designed to determine whether or not the stylar self-incompatibility function can recover after heat inactivation. The effectiveness of heat treatment of pre-anthesis styles for inactivating self-incompatibility was investigated. Finally, we have used the heat-induced inactivation of stylar self-incompatibility competence as a tool for determining whether or not the pollen and style interinteraction and underlying compatible and incompatible pollen tube growth have a reversible or non-reversible influence on pollen tube growth rate.

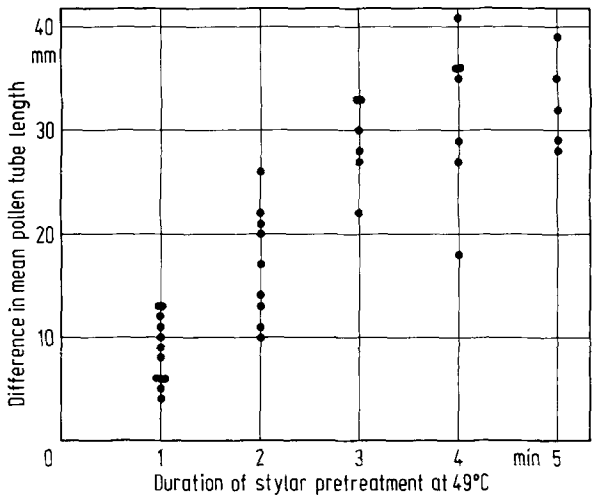

Fig. 1. The increase in the mean pollen tube length in incompatible styles as a function of the duration of pre-pollination stylar heat treatment in 49 °C water. The difference in mean length in treated styles minus the mean length in untreated styles is plotted. Pollen tube length was determined after 48 hours of growth at 24 °C. Each difference is calculated from means based on pollen tube length in 4 or more styles

Materials and Methods

Greenhouse grown plants of cultivars 'Ace' (A), 'Nellie White' (NW), 'Croft' (C), and 'Georgia' (G) provided the flowers. Pollinations were made with fresh pollen obtained from flowers on the day of or the day after anthesis. The effect of heat treatment of the style upon the self-incompatibility reaction was assayed by measurement of pollen tube length in pistils following the procedures developed by Ascher and Peloquin (1966). Pistils were cut from the flowers near the base of the ovary and following pollination they were placed on moistened filter paper in 15-cm petri dishes at 24 °C during tube growth. After 48 hours incubation the pistils were longitudinally bisected with a razor blade and acetocarmine was distributed along the stylar canal. After a few minutes, the excess dye was blotted dry and the stylar canal, as viewed through the dissecting microscope, was gently stroked transversely with a dissecting needle in order to determine the location of pollen tube tips. The longest group of six or more tubes in each half-style was measured to the nearest millimeter and the average of two such values taken as the value for the single whole style. All measurements were made from the stigma surface to the tube tips in the style.

Results and Discussion

Kinetics of heat-induced inactivation

The previous finding that immersion of the style of *Lilium longiflorum* in 50 °C water for 6 minutes can inactivate the stylar incompatibility potential (Hopper, Ascher and Peloquin 1967), prompted an inquiry into the relation between treatment duration and extent of inactivation. The experiment used to test this relation involved immersing the pistil in 49 °C water up to the base of the stigma for 0, 1, 2, 3, 4, or 5 minutes. Immediately after treatment the pistils were self-pollinated and incubated at 24 °C. Pollen tube length was determined after 48 hours and this provided the assay for the treatment effect on the stylar incompatibility. Seventeen individual experiments were carried out during a two year period. Due to limited numbers of flowers at some times not all treatment levels were included. However, pistils were assigned to each treatment group in an entirely random fashion.

The progression of the heat-induced inactivation is illustrated in Fig. 1. Increasing the duration of heat treatment up through 4 minutes results in an increasing extent of inactivation. The progressive nature of this dose-response relation indicates that the threshold is at some duration between 0 and 1 minute. This suggests, that whatever is being altered by heat is extremely heat sensitive at the 49 °C temperature. An idea of the approximate rate of the heat-induced inactivation can be gained by plotting the averages of the individual values for each treatment duration presented in Fig. 1. When this is done (not shown), the slope of the linear part of the curve extending from

0 min. to 3 min. indicates a 9 mm increase in incompatible tube length occurring with each 1 minute increase in treatment duration. Normally the difference between compatible and incompatible tube length after 48 hours of growth at 24 °C in styles of 'Nellie White' is 30 to 40 mm. If the value 35 mm is taken as the mean difference, then the rate of heat-induced inactivation of the stylar incompatibility can be expressed on a percentage basis as [(9mm/min.)÷35mm] × 100 = 25.7 %/min. during the first 3 minutes of heat treatment in 49 °C hot water.

Non-recovery of stylar incompatibility competence

In order to determine whether or not the heat-induced inactivation of stylar self-incompatibility competence is reversible, experiments employing a 48-hour delay between heat treatment and self-pollination were carried out. Both detached pistils and pistils remaining attached to the plant were used. For treatment of detached pistils, the pistils were excised from the flower the day of anthesis and immersed in 49 °C water up to the base of the stigma for 5 minutes. Each experiment involved a minimum of sixteen pistils; eight treated in 49 °C water and eight untreated. Four pistils from the treated group and four from the untreated group were self-pollinated immediately after the time of treatment. The remaining four pistils of each group were placed on moistened filter paper in 15-cm petri dishes at 24 °C during the 48-hour delay period, after which they were also self-pollinated. Pollen tube length in all pistils was determined after 48 hours growth at 24 °C.

The same basic 2 × 2 factorial design was used, with minor modifications, when using attached pistils. Treatment in 49 °C water was accomplished by stripping the tepals from day-of-anthesis flowers and immersing the stigma and style up to the top of the ovary. After treatment the stigmas were dipped in 10 % sucrose solution in order to compensate for possible drying of the stigmatic exudate and ensure normal germination of pollen. Pistils which were to be immediately pollinated were then excised from the plant and handled as outlined above, whereas pistils to be self-pollinated 48 hours later remained attached to the plant under 24 °C constant temperature. As above, determination of pollen tube length after 48 hours growth in detached pistils incubated at 24 °C served as the assay for the effect of the heat treatment and the 48-hour delay on the stylar self-incompatibility competence.

The data in Table 1, indicate that no reactivation of the stylar incompatibility occurs in either detached or attached (on the plant) pistils during a 48-hour post-anthesis period following the heat-induced inactivation.

Independence of stylar incompatibility competence and nutritional capabilities

It was also of interest of determine whether or not stylar self-incompatibility expression could be inactivated by heat treatment prior to anthesis, since the timing of anthesis itself appears to have significance in the development of some stylar functions. For example, it is known that the stigmatic exudate which is essential for pollen grain germination does not appear until shortly after anthesis (Myodo 1962). Furthermore, even when stigmatic exudate is applied to pre-anthesis pistils prior to pollination the subsequent growth of compatible pollen tubes is severely retarded (Ascher and Peloquin 1966b; and Ascher 1967). Since the nutritional capacities of the style to support pollen tube growth are apparently not mature until anthesis, it is possible that the timing of anthesis may be of equal significance to the stylar self-incompatibility function.

To test the effect of heat treatment of pre-anthesis styles the following experimental procedure was followed. Pistils were removed from unopened lily buds and those to be treated were immediately immersed in 49 °C water up to the base of the stigma for 4 minutes. Both the heat-treated pistils and the untreated, control, pistils were then maintained on moist filter paper in petri dishes at 24 °C, and the stigmas were self-pollinated at 24 hours after the anthesis of the bud from which they came. Pollen tube length in these pistils was determined after 48 hours of incubation at 24 °C. A control for any effect of pre-anthesis removal was provided by determination of incompatible pollen tube length in pistils removed after anthesis. Removal of pistils from buds was accomplished by simply making a slit in the side of the bud near its base and then transversely cutting the ovary and pulling the pistil out. Such manipulation does not interfere with the normal opening of the bud (Ascher 1967). It should

be noted here that exudate appears normally on such pre-anthesis excised pistils after the anthesis of the bud from which they come. Furthermore, when pollination is delayed until after bud anthesis, the compatible pollen tube growth in pistils removed as early as 44 hours prior to anthesis is the same as in pistils of the same age removed after anthesis (Hopper 1970). The effect of heat-treatment of pre-anthesis styles on

Table 1. Mean pollen tube length (mm) after a 48-hour incubation at 24° C in pretreated and non-pretreated pistils incompatibly pollinated immediately after or 48 hours after the 5-min. heat treatment in 49° C water

Cultivar		Time of pollination following heat treatment	
		Immediately	48 hr. Delay
	Detached Pistils		
A	Treat	74.4 ± .54	73.0 ± .75
	Control	43.6 ± .55	45.9 ± .69
	Difference	30.8	27.1
G	Treat	70.2 ± .86	73.4 ± .95
	Control	46.1 ± .87	45.7 ± .64
	Difference	24.1	27.7
C	Treat	81.2 ± 2.5	81.2 ± 1.38
	Control	51.1 ± 1.3	51.0 ± 1.33
	Difference	30.1	30.2
NW	Treat	71.0 ± .47	70.5 ± 1.50
	Control	48.9 ± .46	51.5 ± .57
	Difference	22.1	19.0
	Attached Pistils		
A	Treat	66.9 ± .67	68.8 ± .70
	Control	40.9 ± .52	43.6 ± .84
	Difference	26.7	25.2
NW	Treat	72.0 ± 1.46	72.8 ± 1.32
	Control	45.6 ± .98	48.2 ± .65
	Difference	26.4	24.6

Table 2. Mean length (mm) of pollen tubes after 48 hours growth at 24° C in pretreated and non-pretreated incompatible styles excised from buds 0, 13, 18, 37, and 39 hours prior to anthesis. All pollinations were made 24 hours after bud anthesis. Pretreated styles were immersed in 49° C water for 5 minutes immediately after being excised from bud and all styles were kept on moist filter paper in petri dishes at 24° C until time of pollination. Seven a.m. on the morning of anthesis was taken as the time of anthesis

Cultivar	Time at which styles were taken from bud	Stylar pretreatment	
	Hours prior to anthesis	Non-Pretreated (Controls)	Pretreated at 49° C, 5 min.
	0 '	43 '' ± .66 '''	76 ± .94
Ace	18	55 ± .79	74 ± .65
	39	54 ± .44	72 ± 1.02
	0	41 ± .52	75 ± .60
Nellie White	13	52 ± .70	71 ± .93
	37	50 ± .85	74 ± .63

' Styles excised from open flower on the day of or the day after anthesis.
'' Each mean is based on values from no fewer than 5 styles.
''' Standard error of the mean.

the stylar incompatibility function, as tested for here, is therefore unconfounded by effects on compatible pollen tube growth.

As is illustrated in Table 2, heat-induced inactivation of the stylar incompatibility is achieved as early as 39 hours prior to anthesis. A comparison of the 48-hour pollen tube length in styles heat treated at 39, 37, 18, 13 or 0 hours prior to anthesis reveals that the heat-treatment given at these different times is of similar effectiveness in inactivation of stylar incompatibility. Since all pollinations were made at 24 hours after bud anthesis the incompatibility function of the style had as long as 63 hours (39 plus 24) hours in the 39-hour pre-anthesis treatment in which to recover if it was able to. Obviously there appears to have been no recovery (compare the incompatible pollen tube lengths in treated styles, Table 2, with pollen tube lengths for NW and 'Ace' in the treated, immediately pollinated styles, Table 1).

From these results it can be concluded that the timing of anthesis and stigma exudate formation is independent of the sensitivity of the stylar incompatibility to heat.

Another interesting aspect of the data in Table 2 is the slightly increased growth of incompatible tubes in untreated styles removed from the bud prior to anthesis compared to the growth of tubes in untreated styles removed after anthesis. Apparently the stylar self-incompatibility response is weakened slightly in styles removed from buds prior to anthesis. The effect is similar whether styles were removed at 13 or 18 hours compared to 37 and 39 hours prior to anthesis. This aspect of the data suggests that the full stylar self-incompatibility competency may be dependent upon continued stylar attachment to the plant during the pre-anthesis period. In terms of the development of the stylar incompatibility expression during floral development, this aspect of the data may be worthy of further investigation.

Reversibility of pollen tube growth mode

In the experiments utilizing heat treatment of partial lengths of styles an attempt was made to determine the reversibility of the pollen and style interactions underlying the differential pollen tube growth in incompatible compared to compatible styles. Normally, at 24 °C, no difference between compatible and incompatible pollen tube length can be detected within the upper 16 mm (approximately 16 hours of tube growth) of the style. The measurable expression of self-incompatibility in terms of differential pollen tube growth rates begins to appear only after the tubes have grown through 16 to 25 mm of the stylar length (Ascher 1967; and Table 3). Furthermore, there is no polarity within the style affecting this relation (Ascher 1967). Beyond about 25 mm of pollen tube and style contact there begins a marked acceleration in growth rate of compatible pollen tubes and a slight progressive decrease in the rate of incompatible tubes (Myodo 1951; Ascher 1967). The question which was asked here is whether this transitional interval is marked by any complete and irreversible pollen and style interactions such that once a specific interaction (compatible or incompatible) occurs, the pollen tube growth rate is destined to be 100 % compatible or 100 % incompatible regardless of the type of stylar environment encountered by the pollen tube further down the stylar canal.

The experiments designed to answer this question involved heat treatments of partial lengths of styles. In the first set of experiments only the upper length of the style and stigma were immersed in the 49 °C water for five minutes. This resulted in a style having a physiologically inactive self-incompatibility potential in its upper length first traversed by pollen tubes, but a physiologically active incompatibility potential throughout its lower length. In the second set of experiments the procedure was reversed; heat treatment being given only to the lower length of the style, thus resulting in a style having an active incompatibility in the upper length, but an inactivated incompatibility potential in the lower length. In both sets of experiments the detached styles were pollinated with genetically incompatible pollen immediately after the heat treatment. Pollen tubes length was determined after 48 or 72 hours growth at 24 °C.

The results are presented in Tables 4 and 5. The data in Table 4 illustrate that pollen tubes which have grown first through as much as 45 mm of a physiologically compatible length of the style (heat-inactivated) still show a depression in growth rate upon subsequently entering a physiologically incompatible length of the style. This indicates that the pollen and style interactions at the basis of the compatible type high growth rate occurring during the first 45 mm through the style do not impose a complete and irreversible

(all-or-none type) influence on pollen tube growth rate.

Data from the second set of experiments is presented in Table 5. It is obvious that pollen tubes can still make a transition from low growth rate to a higher growth rate after traversing as much as 40 to 45 mm of the upper, physiologically incompatible segment of the style and entering the lower, physiologically compatible, heat-treated, segment of the style. This implies that the pollen and style interactions at the basis of the incompatible type low growth rate do not impose a complete and irreversible influence on tube growth rate.

Both sets of data taken together indicate that at 24° C the normal appearance of differential pollen tube growth rates (compatible vs. incompatible) within the upper 16 to 25 mm of the style is not based on pollen and style interactions which dictate an irreversible compatible or incompatible mode of tube growth.

It is interesting to note that in this respect the interaction between pollen and pistil in *Lilium longiflorum* differs from that in the self-incompatible species, *Arabis arenosa* and *Brassica nigra*. Kroh (1966) has shown that in these *Crucifera* species an irreversible activation of genetically compatible pollen by the stigma vs. a lack of activation of genetically com-

Table 3. Mean length (mm) of pollen tubes determined at 13, 18, or 22 hours after compatible and incompatible pollination of one day old pistils of cultivar 'Georgia'. Incubation temperature during tube growth was 24° C

No. of hrs. after pollination	Type of pollen/style combination	
	Compatible	Incompatible
13	12 ' ± .63 ''	13 ± .45
18	22 ± .37	22 ± .50
22	28 ± .48	23 ± .32

' Each mean is based on values from not fewer than 4 styles.
'' Standard error of the mean.

Table 4. Mean length (mm) of pollen tubes after 48 hours growth at 24° C in detached incompatible pistils which were either partially treated or completely treated in 49° C water for 5 min. immediately prior to pollination. Only the upper lengths of the partially treated pistils extending from the stigma surface to a point below on the style were immersed in the hot water. Cultivar - 'Ace'

Top length (mm) of pistil treated at 49° C for 5 min.	Pollen tube length (mm) in pistils		
	Partially treated pistils	Untreated pistils (controls)	Completely treated pistils
20	43 ' ± 1.70 ''	45 ± 2.00	82 ± 1.11
30	47 ± 1.45	44 ± 1.18	
40	61 ± 2.23	46 ± 1.39	79 ± 1.21

' Each mean is based on values from no fewer than 3 styles.
'' Standard error of the mean.

Table 5. Mean length (mm) of pollen tubes after 48 or 72 hours growth at 24° C in detached incompatible pistils which were partially treated in 49° C water for 5 minutes immediately prior to pollination. Only the lower lengths of the partially treated pistils extending from some point below the stigma to the lower cut end of the ovary were immersed in the hot water. Cultivar - 'Ace'

Hours of growth	Top length (mm) of pistil not treated	Tube length in partially treated pistils	Tube length (mm) in untreated pistils (controls)
48	20 - 25	70 ' ± 1.70 ''	43 ± 1.79
72	40 - 45	79 ± 2.16	62 ± 1.19

' Each mean is based on values from not fewer than 6 styles.
'' Standard error of the mean.

patible pollen is the mechanism underlying differential pollen tube growth. Since genetic control of self-incompatibility in these *Cruciferae* species is sporophytic whereas that in *Lilium* appears to be gametophytic, it is possible to speculate that the operational difference between the two in the pollen and pistil interactions may be a characteristic difference between the sporophytic and the gametophytic system.

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Literature

Ascher, P.D.: Self-incompatibility in *Lilium longiflorum:* A theoretical and experimental approach. Doctoral thesis, University of Wisconsin, Madison, Wisconsin, 1967

Ascher, P.D.; Peloquin, S.J.: Influence of temperature on incompatible and compatible pollen tube growth in *Lilium longiflorum*. Can. J. Genet. and Cytol. 8, 661-664 (1966a)

Ascher, P.D.; Peloquin, S.J.: Effect of floral aging on the growth of compatible and incompatible pollen tubes in *Lilium longiflorum*. Amer. Jour. Botany 53, 99-102 (1966b)

Bali, P.N.: Some experimental studies on the self-incompatibility of *Oenothera rhombipetala* Nutt. Phyton. 20, 97-103 (1963)

Bateman, A.J.: The diversity of self-incompatibility systems in flowering plants. Proc. VIII Int. Bot. Congr. Paris., Sect. 10, 138-145 (1954)

Brewbaker, J.: Pollen cytology and self-incompatibility systems in plants. J. Hered. 48, 271-277 (1957)

Hecht, A.: Partial inactivation of an incompatibility substance in the stigmas and styles of *Oenothera*. In: Linskens, H.F. (ed.), Pollen Physiology and Fertilization, pp. 237-243, North-Holland Publishing Co. 1964

Hopper, J.E.: Heat and x-ray induced inhibition of self-incompatibility in *Lilium longiflorum*. Ph. D. Thesis, University of Wisconsin 1970

Hopper, J.E.; Ascher, P.D.; Peloquin, S.J.: Inactivation of self-incompatibility following temperature pretreatments of styles in *Lilium longiflorum*. Euphytica 16, 215-220 (1967)

Kroh, M.: Reaction of pollen after transfer from one stigma to another (Contribution to the character of the incompatibility mechanism in *Cruciferae)*. Züchter 36, 185-189 (1966)

Myodo, H.: On the pollen tube development and growth of the easter lily *(Lilium longiflorum)*. J. Hort. Soc. Japan 20, 37-43 (1951)

Myodo, H.: Experimental studies on the sterility of some *Lilium* species. J. Faculty of Agriculture, Hokkaido Univ. L11, 70-122 (1962)

Pandey, K.K.: Evolution of gametophytic and sporophytic systems of self-incompatibility in angiosperms. Evolution 14, 93-115 (1960)

Rowlands, D.G.: Self-incompatibility in sexually propagated cultivated plants. Euphytica 13, 157-162 (1964)

Sears, E.R.: Cytological phenomena connected with self-sterility in the flowering plants. Genetics 22, 130-181 (1937)

Townsend, C.E.: Self-compatibility response to temperature and the inheritance of the response in tetraploid alsike clover *Trifolium hybridum* L. Crop Sci. 6, 409-414 (1966)

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

Yamada, Y.: Studies on the histological and cytological changes in the tissues of pistil after pollination. Japanese J. of Bot. 19, 69-82 (1965)

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