論　　説

花粉の発芽と花粉管の伸長 VII
— ツバキの花に含まれている花粉抑制物質 —

会　沢　正　義*

THE GERMINATION OF POLLEN AND THE ELONGATION OF POLLEN TUBE VII

Pollen Grain Inhibition Contained in the Flower of *Cameria Japonica*

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Several students have reported so far on the chemotropism of pollen tubes toward various parts of the plant in the same species. For instance, Molish (1893) found that the pollen of *Narcissus tazetta* grew toward the pedicel, while Mascarehas and Machlis (1962) observed that the pollen of *Antirrhinum majus* grew toward the pedicel as well as to the stem. However, the growth of pollen toward the stamen seemed to be at random. Tsao (1949) also observed at random growth in the petal etc. of *Antirrhinum majus*. [1)]

However, there seems to be no report on the inhibition of anther. When I was making an experiment on the germination of pollen grains and elongation of pollen tubes of *Cameria japonica*, I found that the pollen grains of *Cameria japonica* were being inhibited by various parts of the flower, particularly by anther of *Cameria japonica*.

Of the pollen grains of *Cameria japonica* scattered around an anther of *Cameria japonica* on a culture medium, those closest to the anther showed no sign of germination, while those located a little farther from the anther did germinate but the elongation of pollen tubes was inhibited by the anther. Therefore, a study was made to determine the existence of substance inhibiting the growth of pollen grains in the flower of *Cameria japonica*.

1) Inhibitive effect of various flower parts upon germination of pollen grains and elongation of pollen tubes.

The pollen grains of *Cameria japonica* were collected from fresh flower just bloomed, put in a glass bottle with silicagel, and stored in a refrigerator to keep them at a temperature about −15 ℃.

As the pollen grains as old as one year are known to germinate and produce pollen tubes just as newly collected fresh ones, those collected within six months period were used in the experiment.

Samples of flower parts of *Cameria japonica* used were anther, filament, stigma, style, pelal and ovary. They were collected as required.

The large flower parts were cut with cover glass on the slide glass to a size of 5mm×5mm in the

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contact surface for agar plate.

A culture medium containing 10% saccharose and 1% agar was employed. Heated saccharose agar solution was put on the slide glass to form a layer about 2mm thick. When it solidified, the pollen grains were sown in a straight line on the surface of the agar plate with cover glass.[2)]

A part of the flower was placed one mm apart from the line of pollen grains. The pollen grains and a part of the flower on the slide glass were placed in a humidified Petri-dish and cultivated at 30 °C. After about 20 hours, the reaction of pollen grains against a part of the flower was observed microscopically. The average length of the pollen tubes was observed through a projector Cannon Slide&Ster 300, and recorded as shown in Figure 1. The results are summarized in Table 1.

These results reflected that the pollen grains of *Cameria japonica* are inhibited by the surface and cut section of anther as well as of ovary, and by the cut section of petal of *Cameria japonica*.

The pollen grains laying closest by these material did not germinate, while those laying a little farther did germinate but the elongation of pollen tubes was inhibited.

It was further observed that inhibited pollen tubes stopped their growth with the protoplasma ceasing its streaming. The tips of the pollen tubes were broken and the protoplasma was ejected, thus killing the cells completely. The pollen grains laying farther from a part of the flower were of coures alive and growing and the plasma streaming was observed.

However, the anther before anthesis did not manifest any inhibitive function. In other words, no inhibitive substance was observed on the surface of that anther, but if it is thermally processed, it manifests a prohibitive function. (This is an unpublished theory).

From this fact, I theorize that, as Miki[3)] and Rosen[4)] stated, the plant body contains both inhibitive and promotive substances, and that heating may dissolve promotive substance, while drying of the anther and ovary and cut section of petal after anthesis may kill the function of promotive substance thus surfacing certain inhibitive function. This would account for the phenomena that the anther, the surface of ovary, and the cut section of petal come to possess certain inhibitive function after anthesis. However, the reasons why wet cut section of ovary always manifests an inhibitive function and why the anther produces certain substance which inhibits the growth of pollen grains are not known yet.

2) Inhibitive substance contained in the anther prevents the germination of pollen grains and elongation of pollen tubes.

As mentioned above, the anther prohibits the growth of pollen grains. Therefore, it was used to study the inhibitive functions. The pollen grains and anthers were collected from blooming fresh flowers and stored in a refrigerator with silicagel to keep it at −15 °C. They were used for experiment within a six month period after collection. It was assumed that the anthers had a uniform size for experimental purposes. A culture medium containing 10% saccharose and 1 % agar was employed.

The pollen grains were sown straight on the surface of agar using cover glass. The anther was placed in the middle of a pollen grain line and cultivated at 30°C in a humidified chamber to check inhibitive function exerted on the growth of pollen grains. The results are shown in the upper photograph of Figu-

re 2, which shows that most pollen grains laying closest by the anther did not germinate.

Further, the pollen grains were sown straight and cultivated for five hours, and then a anther was placed near elongating pollen tubes and the cultivation continued 15 hours, after which an observation was perfomed. The results are shown in the lower photograph of Figure 2. In this case, the elongation of pollen tubes was stopped and as in the preceding cases, the tips of the pollen tubes were broken and the protoplasma ejected.

Therefore, it was ascertained that the inhibitive substance contained in the anther prevents both the germination of pollen grains and the elongation of pollen tubes. However, in order to determine which of the germination of pollen grains and elongation of pollen tubes is more strongly affected by the inhibitive substance, the following experiment was conducted.

3) Differences in inhibitive power as related to the distance between the anther and the pollen grain.

As mentioned above, it was established that the anther surely inhibits both the germination of pollen grains and the elongation of pollen tubes. Therefore, the distance between the anther and the pollen grain line was changed to see the degree of inhibition exerted upon the germination of pollen grains and the elongation of pollen tubes and also to see which of the germination to pollen grains and elongation of pollen tubes is more strongly affected.

The experimental methods and material were the same as for previous experiments. An anther was placed at a certain distance from the pollen grain line on the surface of the culture medium and the reaction of pollen tubes toward the anther was observed after 20 hours cultivation.

As shown in Figure 3, the more the anther is placed close to the pollen grain line, the more it exerted inhibitive influence. Most pollen grains located within a range of one mm from the anther showed no sign of germination and those at a 5mm range had only about 2mm long pollen tubes.

The inhibitive influence was exerted even on those pollen grains located as far as 20 mm from the anther and elongation of pollen tubes were inhibited. In other words the inhibitive power was proportional to the closeness of pollen grains to the anther and the density of inhibitive substawe It was also observed that the pollen tubes extended toward the anther immediately after germination changed these courses of growth away from the anther as soon as the tube tips came close to the anther. [5)]

The pollen grains laying 20 mm away from the anther were observed to have extended 12mm-13mm long pollen tubes, about the length of the control, toward a direction opposit to the anther when viewed from the line of pollen grains, the symmetric axis. It the inhibitive substance of the anther had worked on the pollen grains themselves which was 20mm-21mm away from the anther (the width of the pollen grain line was 1mm), the length of pollen tubes extended toward the opposit side of the anther should have limited to about to about 8mm, the length of the pollen tubes on the same side of the anther. It appears, therefore, that the inhibihive sudstance works more strongly on the pollen tubes than on the pollen grains themselves. Further, it seems to work in particular, on the growing tips[2)] of the pollen tubes, as the tubes, bend themselves and turn away from the anther if these near the anther.

Figure 1. As pollen grains were sown straight, the pollen tubes elongated about straight. (control)

Upper Diagram :

Shows an average length of pollen tubes by a line connecting the tips of pollen tubes.

Lower Diagram :

Graphically represents the length of pollen tubes as shown above.

P : A line of pollen grains.

Pt : Pollen tubes.

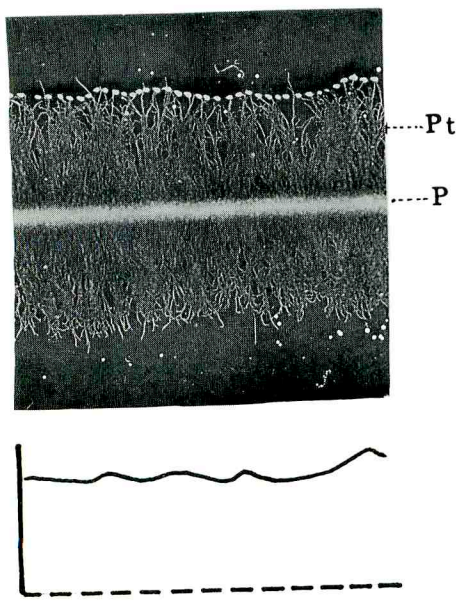


Table 1. Inhibitive methods applied to the elongation of pollen tubes by various parts of *Cameria japonica*.

S : Surface. CS : Cut Section.

O : The tips of pollen tubes formed a line parallel to the line of pollen grains.

−1 : Inhibition

−2 : Stronger Inhibition.

−3 : Strongest Inhibition.

◌ : Position of the anther etc.

P : Line of pollen grains.

Pt : Elongated pollen tubes.

	anther		filament	stigma	style	ovary		petal	
	S	CS	S	S	S	S	CS	S	CS
before anthesis(bud)	0	−2	0	0	0	0	−1	0	0
the day of anthesis	−1	−2	0	0	0	0	−1	0	0
directly after anthesis	−3	−3	0	0	0	−1	−2	0	−1
one year after anthesis	−2	−2							

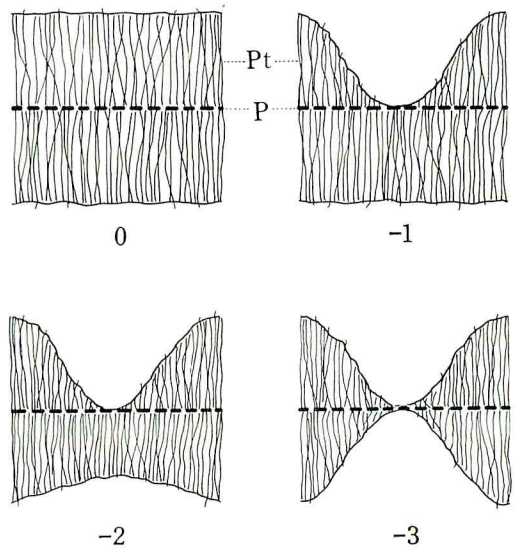


Figure 2. Inhibitive substance contained in the anther prevents the gervents the germination of pollen grains and elongation of pollen tubes.

Upper Photograph :

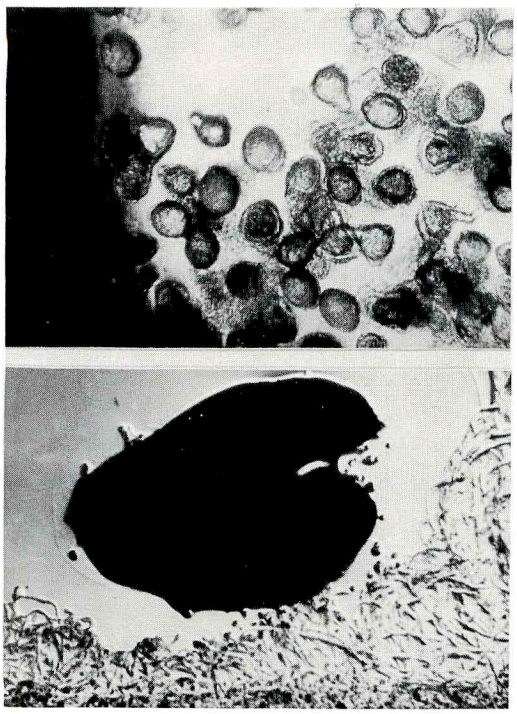
The pollen grains laying closest by the anther did not germinate almost.

Lower Photograph :

The elongation of pollen tubes was inhibited by the anther.

The pollen tubes extended toward the anther immediately after germination changed these courses of growth away

from the anther as soon as the tube tips came close to the anther.



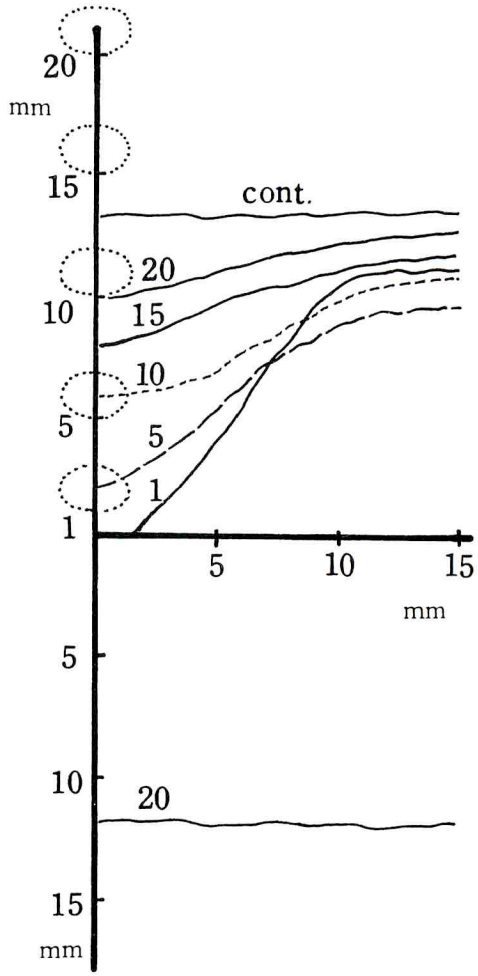


Figure 3. The distance between the pollen grain lead line and the anther and inhibihion. The curve represents a line connection the tips of pollen tubes.

1—20 : Represents a distance in m. m. between the line of pollen grains and the anther.

◌: Position of the anther.

Summary

This study covers the observation of the influences exerted by various parts of flowers of *Cameria japonica*, particularly by the anther, in various development stages, upon the germination of pollen grains of *Cameria japonica* and elongation of pollen tubes cultivated by an artificial medium.

1) The results of above obsevation are show in Table 1, which indicate that the growth was either

inhibited or not affected, but there was no promotive influences.

2) The inhibitive function was observed in the anther, ovary, and petal.

3) The surface of the anther after anthesis, and cut sections of the anther in all atages of growth manifested an inhibitive function.

4) The surface of ovary after anthesis and the cut sections of the ovary in all stages of growth showed inhibition.

5) The cut section of petal after anthesis showed inhibition.

6) The surface of filament, stigma, and style did not exert any influence in all stages of processes.

7) The inhibited pollen tubes stopped their growth and plasma streaming came to a standstill. The ejection of protoplasma was observed at the tips of pollen tubes.

8) The inhibitive substance contained in the anther worked more strongly on the elongating part of pollen tubes than on the pollen grains themselves.

9) The pollen tubes showed a negative chemotropism in relation to the anther.

10) The pollen grains within a range of one mm from the anther showed almost no sign of germination, and those at a 5mm range could elongate their pollen tubes noly up to 2mm. Even the pollen tubes of pollen grains located about 20mm away received an inhibitive influence.

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