THE REACTION OF THE STIGMATIC TISSUE AGAINST POLLEN-TUBE GROWTH IN SELLED SELF-SterILE PLANTS

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In earlier papers I have pointed out an analogy between the reactions taking place in styles of selfed self-sterile plants of *Nicotiana Sanderae* and the antigen-antibody reactions which occur in animals. Some recent investigations of pollen-tube growth indicate that this analogy is more precise than had previously been supposed.

In 1917, East and Park\(^1\) reported a number of studies of pollen-tube growth rates in this species and in *N. alata*. The method used was to pollinate a series of comparable flowers on a given plant with their own pollen or with pollen from another plant at as nearly the same time as possible, and then to fix, section and stain the styles at successive intervals. Though it was found that there was some difficulty in arriving at a correct estimate of the average distance traversed at any given time, owing to a normal variation of several hours in the time required for the germination of the pollen grains and to the fact that the pollen tubes frequently swell markedly at the ends and stop growing, it was found possible to determine several fairly accurate curves by taking points representing the modal condition of the most advanced cohort of tubes in each case. Since the sections of a single style would show a hundred or so tubes grouped thus within a distance of one millimeter from each other, it was assumed that these were the effective pollen tubes.

The results were consistent in that each curve from an incompatible mating approached a straight line having an increment varying between 1 mm. and 3 mm. a day, while each curve from a compatible mating exhibited a rather constant acceleration.

Although the pollen-tube growth-curves from the incompatible matings are fairly described by saying that they "approached" straight lines, as a matter of fact there is indication (see Fig. 3 in the paper cited) that a depressive influence becomes operative during the early growth of the tubes which brings about a slight change in the trend.
In addition to these two distinctive types of pollen-tube growth manifested under normal conditions, it has been found that the fertility of incompatible matings is increased (a) during the last week or so of the flowering season and (b) by pollination of the young flower between 24 hours and 48 hours before it is due to open. Seeds have been obtained through "end-season" fertility in over half of the various combinations of the fifteen known allelomorphs of the self-sterility gene S which have been investigated. Seeds have also been obtained by bud pollination from all the combinations except S1S4, S1S6, S4S4 and S5S5.

Naturally one asks the question: Why can these self-incompatible plants be selfed in the bud, or at the extreme end of the flowering season, and not in the open flower or during the height of the flowering season?

![Pollen-tube growth curve of S1S8. O is for mature flower; B is for flower pollinated in bud 24 hours before opening. Points probably due to growth of S8.](image)

It was thought that a more careful study of pollen-tube growth, as shown in smears and sections of the pistils fixed at given times after pollination, might give the answers to these questions and thus throw some light on the physiological reactions involved. In furtherance of this plan, all the paraffin section methods and smear methods mentioned in the literature as being suitable for pollen tubes have been tried by my technical assistants, but without conspicuous success. The earlier slides, stained with safranin by East and Park, were satisfactory. Unfortunately, however, it has been impossible to duplicate the results then obtained. The slides studied at that time were stained with a German safranin product purchased before the World War, and no comparable results have been obtained from the various lots of safranin available since. The best results have been ob-
tained with sectioned material stained with 0.5 per cent thionin in 20 per cent alcohol. This stain brings about an excellent contrast in the short abortive tubes, but does not serve so well in differentiating normal tubes. Nevertheless, it was found that it was possible to detect the ends of the tubes if the slides were studied slowly and carefully under a magnification of 600 diameters.

About one thousand slides prepared by this method were studied by the writer and by Miss Estella Humphrey, who prepared the greater part of them. Genotypes used were \( S_1S_2, S_1S_3, S_1S_4, S_1S_6, S_1S_7, S_1S_8, S_1S_9, S_1S_{10}, S_1S_{15}, S_1S_{1}, S_2S_2, S_7S_7 \) and \( S_{10}S_{10} \). In some instances, growth curves were obtained from several plants; and in the case of \( S_1S_{15} \), three clones were studied. In my opinion, there is adequate evidence for the following conclusions. The observations upon which they are based are numerous and consistent. The three curves shown are representative samples.

1. In the self-sterility genotypes which are self-fertile in the bud—and by this term it is meant to include only pollinations made 24–48 hours before the flower is due to open—the growth curve of the pollen tubes is a straight line. In flowers from these same plants which have been selfed just after the flower has opened, the pollen-tube growth-curve is depressed after a growth of 8–10 millimeters. The depressive effect (see Figs. 1 and 2) is so marked that fertilization cannot take place within the usual "life" of the flower. The time that the flower remains on the plant is ordinarily 5 days, and is seldom more than 7 days.
The most plausible inference from these facts is that there are substances present in the stigmatic tissue of the mature flower which react with substances present in the pollen tube, thus causing pollen-tube growth to be inhibited. These substances are not present in the bud. They appear sometime during the twenty-four hour period preceding the opening of the flower. As the pollen passes this zone of interference in the stigmatic tissue, the effect tends to be lost. The growth of the pollen tubes again approaches a straight line trend, but at a slower growth rate.

2. In self-sterility genotypes which are not self-fertile in the bud, both the bud pollinations and the open flower pollinations have pollen-tube growth-curves which exhibit similar depressive changes in trend while passing this same stigmatic zone of interference (Fig. 3).

One infers that in these genotypes the inhibiting substances are developed earlier.

3. In nearly all the growth-curves comparing self-pollinations of open flowers and of buds from the same plant (Fig. 1 is typical), the growth rate of the pollen tubes in the mature flower is more rapid than the growth rate in the bud, previous to passing the interference zone.

The cause of this phenomenon appears to be the more satisfactory supply of nutrients by the mature flowers during the period before the incompatibility reaction occurs.

4. End-season fertility of self-sterile plants is presumably due to the fact that during the last week of the flowering season the plants are unable to produce adequate amounts of the substances which cause the incompati-
bility reaction. In this case the pollen-tube growth-curve of selfed self-fertile plants, even on mature flowers, is essentially a straight line. Only the slightest indication of a depressive effect at the interference zone is shown.

5. Slides made at successive periods after very young buds have been selfed, buds which are not due to open for 72–96 hours, show that the pollen grains fail to germinate. The tissue of the stigma is quite immature. The cells contain no cytoplasmic substances stainable in thionin. About 48 hours before the flowers are due to open, however, globules of substances staining reddish brown with thionin appear within the cells of the stigmatic tissue. These substances begin to diffuse out of the cells about the time the flowers mature. It is suggested that these substances are connected with the incompatibility reaction under discussion. The change in the trend of the growth curves is coincident with their diffusion from the cells to the intercellular spaces. I have been unable to determine their nature.

6. Evidence has already been presented2 (East, 1934) which indicates that the two major influences affecting pollen-tube growth are (a) a nutritive reaction, and (b) a mutual reaction between style and pollen tube resembling a reaction of the antigen-antibody type. The second reaction is the incompatibility reaction. The pollen-tube growth studies made here corroborate the previous finding.

7. If it be granted that the constant growth of the pollen tubes in bud pollinations is illustrative of a purely nutritive reaction, then these growth-curves represent a new type. There is some acceleration, naturally, after the pollen grains germinate; but from a period of about four hours after pollination, the growth is constant. There is no accumulation of depressive factors. In all other growth studies of which I am aware, such cumulative depressive factors put in an appearance earlier or later, and cause the growth curve to take the logistic form first described by Verhulst.
