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THE GERMINATION OF TOMATO POLLEN ON THE STIGMA
(as an aid to the study of fruit setting probems)

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First a short description of how the germination of pollen on the stigma is examined, will be given. After that attention will be paid to four items of the fruit setting problem.

1. METHODS

A modified staining method after Dionne and Spicer (a squash technique) gives very good results. [1,4]. With this method the tissue of style and stigma stains red, while the grains of pollen turn to dark blue.

The germination of pollen can best be examined by administrating the pollen to the stigmas of tomato flowers, castrated a day before, and making preparations after a certain time. One hour after pollination all grains of pollen are blue, just as the germination-tubes. After two hours a larger number of grains has germinated, while the germination tubes have become considerably longer and have turned to a lighter blue. They then still contrast clearly with the red tissue of the style and the stigma. As the germination advances the picture becomes less clear since the germination-tubes first and the grains afterwards lose their blue colour and change to light red. The percentage of germination reaches a maximum after about 5 hours. Only the ungerminated and the last germinated grains of pollen are still blue.

In this way considerably higher and besides more constant germination-percentages are found, than with germination in vitro (Table 1). However the figures thus found are probably too favourable. During fixation and hydrolysation, part of the non-germinated grains may be washed away from the stigma. In our opinion the percentages found after two hours are the most reliable.

TABLE 1. — Germination in vivo and in vitro

	Germination percentage of the pollen		
	in vivo		in vitro
	after 2 hours	after 5 hours	after 5 hours
22nd April 1960	75.4	84.9	45.0
28th Oct. 1960	71.6	87.1	17.8
16th June 1961	74.4	86.6	33.2

2. Castration and transfer of flowers to nutrient-mediums

It is desirable to castrate the flowers beforehand in order to prevent the results from being affected by pollen produced by the plant itself (the tomato is mainly a self-pollinator). By castration the stigma is more exposed to evaporation. It was therefore important to determine to what extent castration can affect the germination results. Male-sterile plants have been used for this.

Transfer of flowers to a nutrient-medium is, among others, of importance for the study of the germination of the pollen in abnormal flowers, as these will hardly ever occur under comparable growing-conditions. As nutrient-medium an agar has been used on which tomato fruits can be cultivated in vitro [3].

Table 2 shows the average results after two hours of some of our experiments. The number of grains of pollen which stick to the stigma appears to be considerably larger on the flowers placed in the nutrient-medium. This is probably so because the flowers in the nutrient-medium stand erect while the flowers on the plant often hang down more or less. In the last case the pollen falls off more easily. On the plant as well as on the nutrient-medium non-castrated flowers show a germination-percentage about 10 % higher than that of castrated flowers. The germination-figure in the case of the nutrient-medium was nearly 10 % lower than on the plant. This was partly due to the use of too thin a layer of nutrient-medium on which in particular the castrated flowers showed many drying symptoms (as a result, less pollen stuck to the stigmas of these flowers). These figures reveal at any rate that the results obtained with castrated flowers and flowers on nutrient-medium are very useful.

Table 2. — Germination in castrated flowers and on a nutrient-medium

Treatment	Germination of pollen after 2 hours	
	number of grains per flower	percentage
uncastrated, on the plant	170	81.7
castrated, on the plant	176	67.6
uncastrated, on nutrient-medium	511	70.8
castrated, on nutrient-medium	245	59.5

3. Germination of the pollen in defective flowers

Highly defective tomato flowers of which in particular the corolla and the stamen have been greatly reduced are called in Dutch « gerstebloempjes » (« barley-flowers »). The germination of pollen in these defective flowers and in normal flowers has been compared. Both types of flowers have, or have not been vibrated with the American truss-vibrator. Also, part of the defective flowers has been pollinated with pollen of normal flowers. Pollen-germination has been examined both in flowers on the plant and in flowers on a nutrient-medium. These were placed in the same glasshouses as the plants with normal flowers. The experiment was made twice at the end of March. Both times

vibration and pollination was started early in the afternoon and the germination of pollen was checked two hours later. The average results are shown in table 3.

TABLE 3. — Germination in defective flowers :« gerstebloempjes » (« barley flowers »)

Treatment	Germination of pollen on the plant		Germination of pollen on nutrient-medium	
	number of grains per flower	percentage	number of grains per flower	percentage
defective flowers, untreated	0		0	
defective flowers, vibrated	0		0	
defective flowers, pollinated	124	97.0	175	95.2
normal flowers, untreated	64	93.8	320	96.0
normal flowers, vibrated	244	96.6	301	97.7

It is first of all noticeable that pollen is never found on the stigmas of the defective flowers. It is therefore highly remarkable that the good pollen of a normal flower has the same germination-percentage on the defective flowers as on its own flower. Neither was this percentage influenced by the transfer of flowers to a nutrient-medium.

4. INFLUENCE OF CLIMATIC FACTORS ON THE GERMINATION OF POLLEN

At the beginning of our experiments we tried to learn something about the influence of climatic factors by examining the germination of pollen in greenhouses under different weather conditions. However such observations are hard to interpret, since various climatic factors change silmultaneously. Yet they indicate that a high air-humidity is not only detrimental to the loosening and distribution of pollen but also to germination.

Table 4 shows the germination-percentages on the same tomato crop during a few days with variable weather conditions. On a sunny day with moderate air-humidity a

TABLE 4. — Germination of pollen under different weather conditions

Weather condition	Germination percentage	
	on the stigma after 2 hours	in vitro
cloudy (very high air humidity)	30.2	14.2
cloudy (rather high air humidity)	74.4	33.2
sunny (moderate air humidity)	86.9	48.9

germination-percentage was found three times as high as on a cloudy day with a very high air-humidity. It is remarkable that the germination-figures in vitro show entirely the same line, though its level is only about half as high. This could indicate that in overcast weather with high air-humidity the grains of pollen formed are less vital.

The course of the various climatic factors (exposure to light, temperature, air-humidity) on these days has been compared with the course of the germination of pollen. There was a rather clear connection only between the relative air-humidity and the germination of pollen figure 1.

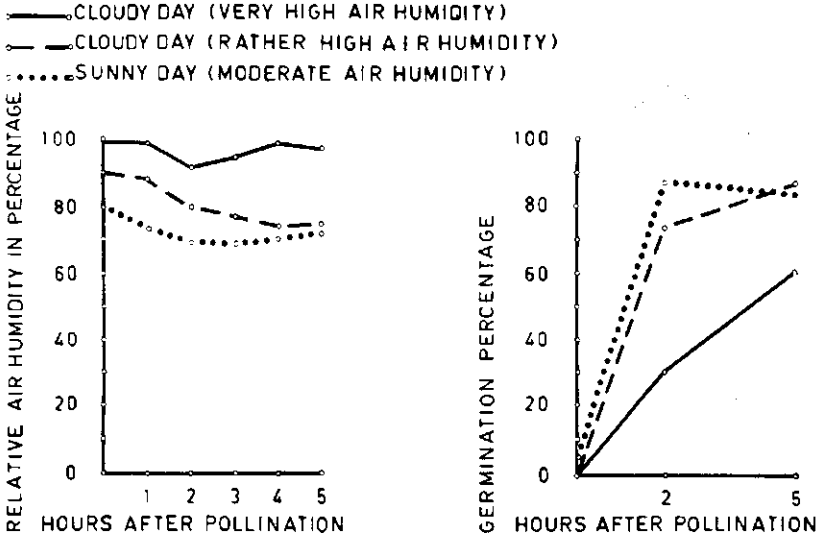

FIG. 1. — Relative air humidity and germination of the pollen

On the above-mentioned sunny day investigations were made as to the effect of sprinkling and shading on the germination of pollen. From table 5 it appears that the germination-percentage decreased by shading and still more by sprinkling. So unnecessary sprinkling and shading may be harmful for the germination of pollen. The sticking of pollen to the stigma may have improved slightly because of these measures, considering the somewhat larger number of grains found on the stigma. In order to determine more accurately, the influence of temperature and air-humidity on the germination of pollen tomato flowers on nutrient-mediums were placed in a set of temperature-chambers. In the various sections of this thermostat, temperatures were fixed at 7°C difference, ranging from 7° to 35°C. In addition to this we worked with air-humidities of 50 to 90 %.

TABLE 5. — The effect of shading and sprinkling on germination of pollen

Treatment	Germination percentage after 2 hours	Total number of grains per flower
normal	86.9	76
sprinkling	63.0	84
shading	75.6	82
sprinkling and shading	63.4	88

Some results are shown in figure 2. At 7°C the germination seems to be practically nil. Between 7 and 21°C the rate of germination after 2 hours increases almost rectilinearly with the temperature. The optimum lies at about 25°C. Judging these figures, one has to realize, that at high temperatures the germination-speed is much higher

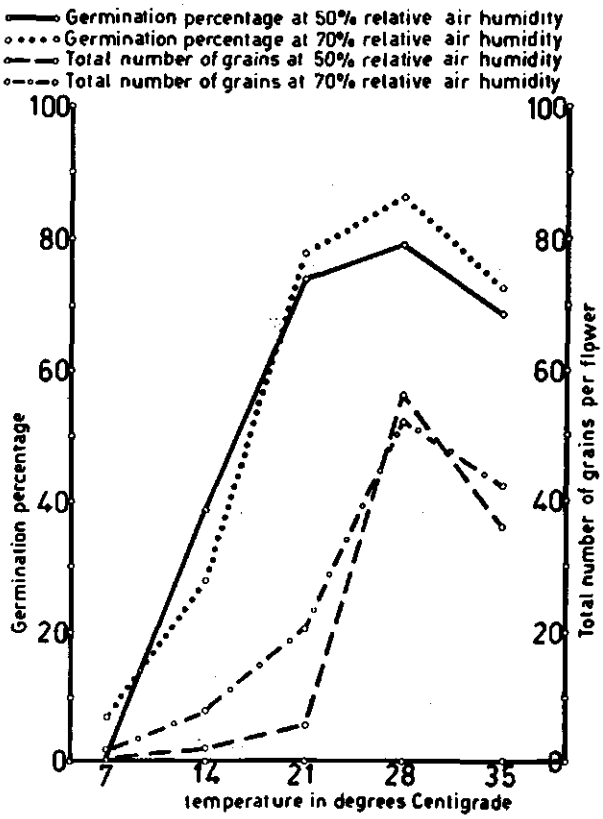

FIG. 2. — Germination and sticking of the pollen to the stigma at different temperatures and air humidity degrees

(optimum at about 30°C). With regard to the rates of air-humidity, especially the lower ones (50 %) interest us, as these low air-humidities did not occur in our germination-tests in greenhouses. At high temperatures the germination of the pollen seems to have suffered slightly from low air-humidity. In greenhouses this disadvantage might have been greater because of direct solar radiation.

The total number of grains of pollen found on the stigmas is also shown in figure 2. The speed of germination may have been of importance here. Rapid germination causes the germination-tube to enter the style sooner which prevents the falling down of the grains both during germination and preparation. At low air-humidity (50 %) the sticking of the pollen has been considerably less at all temperatures, except at 28°C, where germination goes very fast.

5. Vibration and pollination

After its introduction to the Netherlands the American truss-vibrator rapidly made its way into the nurseries. The truss-vibrator is used on tomatoes for shaking the pollen

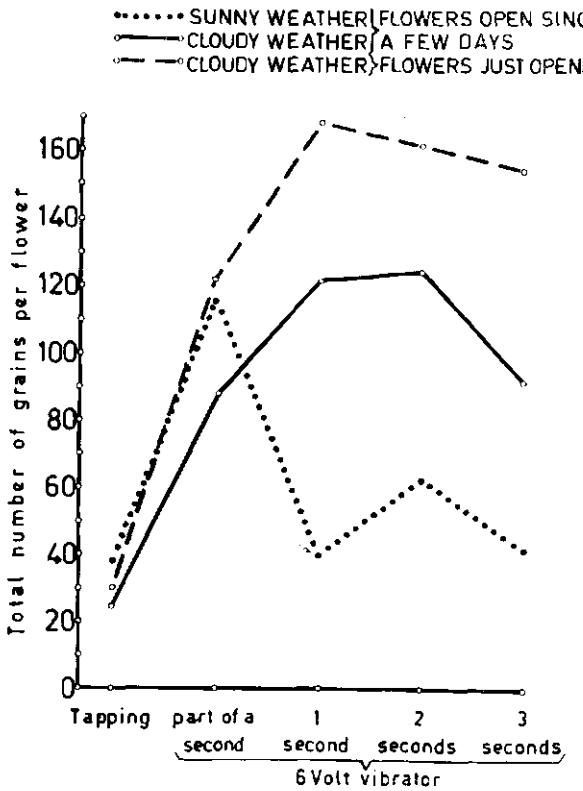

Fig. 3. — The effect of duration of truss vibration on the sticking of pollen to the stigma

from the anthers thus improving distribution. One could imagine that on sunny days the surface of the stigma is less sticky and that part of the pollen is shaken off the stigma by vibration. The effect of truss-vibration has therefore been investigated under various weather conditions. The flowers were not castrated.

First of all the effect of the duration of vibration was investigated. The check-plants were moved by tapping the strings with which the plants were fastened. In one treatment the plants were vibrated during a part of a second (the trusses were only slightly touched). Other plants were vibrated during one, two and three full seconds. According to expectations these treatments hardly affected the germination percentage. However, striking differences in the quantities of pollen on the stigmas (figure 3) became apparent. By tapping the strings only, little pollen landed on the stigma, irrespective of weather-conditions. In sunny weather, short vibration of the trusses gave by far the best results (about three times as many grains of pollen on the stigma as by tapping). By longer vibration of the trusses in sunny weather, part of the pollen obviously fell off the stigma, reducing the pollen coverage to the same amount as with the tapped plants. In cloudy weather both short-time vibration and longer vibration gave favourable results. The best sticking of pollen to the stigma was obtained when vibration lasted for 1 or 2 seconds (5 to 6 times as many grains as by tapping). The sticking of pollen in flowers just open was slightly better than in flowers which had been open for a few days.

Secondly, the effect of the intensity of truss-vibration was investigated. The same vibrator was therefore connected to 4 and 6 volt batteries. During this investigation vibration lasted two seconds. This may have been too long for both voltages in sunny weather, considering the relatively low pollen coverage of the stigmas (figure 4).There

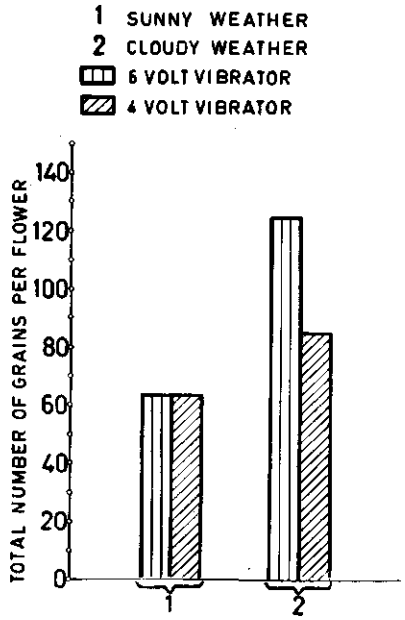

FIG. 4. — The effect of intensity of truss vibration on the sticking of pollen to the stigma

was no difference between the two voltages. In cloudy weather the sticking of pollen to the stigma was better and a considerably better pollination was achieved, if the vibrator has been connected to a 6 volt battery. A new method of working was based on these experiences. The effect is a saving of time, while still an optimal fruit-setting is obtained [2].

6. Virus-infestation and fruit-setting

The tomato mosaic-virus (Lycopersicum virus nº 1) is widely spread in Dutch tomato-growing under glass. In the initial period of virus-infestation we often notice a wilting of the leaves during sunny weather. The setting of fruit in the trusses which develop in this period is poor. In order to study the sticking to the stigma and the germination of pollen in plants affected by virus, groups of tomato-plants have been inoculated with tomato mosaic-virus at intervals of 5 days or one week. Thus at the same moment and under equal conditions the germination of pollen could be investigated at various periods after inoculation.

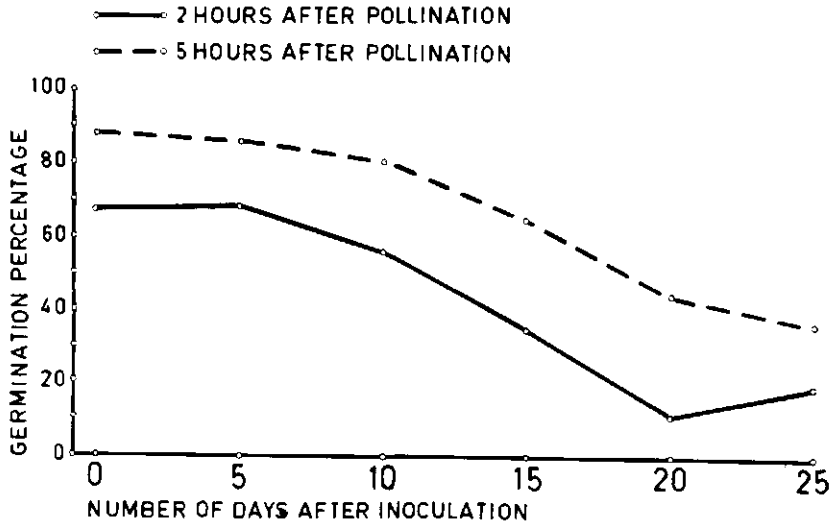

Fig. 5. — Germination percentage at different stages of virus infestation

Even he first experiment gave a striking result (figure 5). Until ten days after inoculation no effect of virus-infestation on the germination of pollen was noticeable. After that, the germination-percentage decreased rapidly and reached at about 20 days after inoculation, a minimum, which was most clearly expressed in the percentage after two hours.

This experiment was repeated several times. The observations were extended over a period twice as long as in the first case. Slight virus symptoms only occurred and the leaves did not wilt. Accordingly the germination-percentages were less clearly affected

by virus-infestation than during the first experiment. However, a considerably decreased sticking of the pollen to the stigmas was noticed (figure 6). From 2 to 6 weeks after inoculation only a small fraction of the normal number of grains of pollen was found on the stigma.

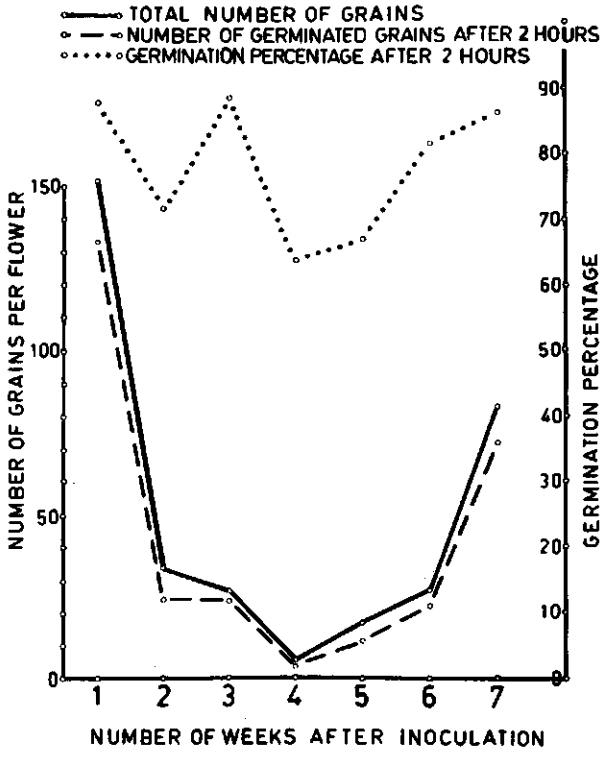

FIG. 6. — Germination and sticking of pollen to the stigma at different stages of virus infestation

It seems justified to draw the conclusion from these data that with a moderate virus-infestation the poorer fruit-setting may be met for a considerable part by improving the conditions for the sticking of pollen to the stigma.

7. CONCLUSIONS

By investigating the germination of the pollen on the stigma a better information about the fruit-setting problems of the tomato is obtained. Various stages of pollination can be distinguished which all may be of great importance for the fruit-setting. These stages are :

Environment

a. Production of pollen.
b. Liberation and distribution of pollen.
c. Sticking of the pollen to the stigma.
d. Germination of the pollen and penetration into the style.
e. Penetration of the pollen tube into the ovules.

The production of pollen (phase a) in highly defective flowers is nil. The liberation of the pollen (phase b) during humid dark weather leaves much to be desired, but can be improved by making a correct use of the truss-vibrator. The sticking of the pollen (phase c) is, however, unfavourably affected by dry and sunny weather, by virus-infestation and by a too intensive vibration of the trusses. The pollen germination (phase d) is poor in dark, humid weather and when virus is present. Both the degree and the speed of germination are largely dependent on the temperature. About the last stage, we are insufficiently informed.

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DISCUSSION

Q. — de BEER (The Netherlands) : Do you think that the germination in vitro can give you an indication of the possible fertilization percentages ?

R. — Indeed it can, if you apply the best method, but germination on the stigma gives better information.

Q. — KADINOPOULOS (Greece) : What percentage of sugar have you used in vitro germination of the pollen ?

R. — I have used seven percent of sugar.

Q. — Have you used stigma extracts in those germination tests ?

R. — No.

Q. — In that case it seems difficult to compare the results in percentage of germination in vitro and in vivo.

R. — Yes but we have checked several methods for germination in vitro and we choosed the best one for comparing with the germination on the stigma.