The Effect of Flower Age, Time of Day and Variety on Pollen Germination of Onion, *Allium cepa* L.[1]

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Abstract. Onion pollen of 2 different parents, B12115C and B2215C, was collected at 9:00 AM and 2:30 PM from flowers at approximately 1, 3, and 6 days after anthesis. This was germinated in hanging drops of a sucrose-gelatin medium. The percentage of pollen germination from B12115C was significantly higher than the percentage from B2215C. There was no significant difference in germination between pollen collected and germinated in the morning versus pollen collected and germinated in the afternoon. The results also show a linear relationship between pollen age and pollen germination. The ability to germinate declined rapidly after the first day and approached zero by the sixth day after anthesis. Hand pollination and style studies indicate that onion pollen tubes are capable of growing the entire length of the style, a distance of approximately 4 mm, in 24 hr.

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Introduction

IN the commercial production of hybrid onion seed, the pollen parent, C-line, is interplanted with the male-sterile seed parent, A-line. Because the seed parent produces no pollen, emasculation is not necessary and the production of hybrid onion seed is economically feasible. However, in the past few years low seed yields in the onion seed industry, particularly among hybrids, has become a major problem. Factors which may affect the seed set of onions include the comparative blooming dates of both parents, the ratio of pollen rows to seed rows, bee activity, weather conditions during bloom, and the duration of receptivity of the stigma (9). In addition, the pollen longevity is an important factor because pollen viability is a prerequisite to fertilization and seed set.

Several factors may affect the viability of the fresh pollen. The age of pollen affects the percentage of germination and the seed-set ability of certain pollens. Maximum germination of various fruit and vegetable pollens occurs on the day of flower anthesis or anther dehiscence (5, 6). The germination percentages of pollen taken from *Solanum* and *Capsicum* flowers 1 or 2 days before anthesis and pollen from closed or abscissed flowers was very low (5, 10). Dempsey (2) observed that maximum fruit set in pepper was obtained with pollen collected on the day of anthesis. Pollinations with pollen collected 1 day before or after anthesis resulted in reduced fruit set and seed production, and pollinations with pollen collected 2 days prior to anthesis or 3 days after anthesis were not successful.

The time of day of anther dehiscence and pollen collection also may affect seed-set ability of pollen. Adlerz (1) reported that the percentage of fruit set in watermelon was higher following pollination between 8 to 9 AM than between 9 to 10 AM, 7 to 8 AM, or 6 to 7 AM. Morning pollination with stored sorghum pollen produced more seed than afternoon pollination (11). Eršov and Vorob'eva (4) found that pollinations in onion, both within and between varieties, at 10 AM or 6 PM resulted in a higher seed set than pollinations at 2 PM.

The work reported here was designed to outline the influences of various factors on the germination of onion pollen.

Materials and Methods

Preliminary studies using various levels of sucrose, boron, and calcium were conducted to find the individual medium requirements of onion pollen. The resultant selected medium was a modification of one proposed by Kwack (7) consisting of 10% sucrose, 100 ppm H_3BO_3, 300 ppm $Ca(NO_3)_2 14H_2O$, 200 ppm $MgSO_4.7H_2O$, and 100 ppm KNO_3. This medium was modified by adding 1% gelatin to increase the viscosity.

The pollen and the medium were thoroughly mixed by stirring with a glass rod, and a drop of this mixture was placed in a petri dish which had a grating etched into the bottom (Fig. 1). Thirty-six separate hanging drops were placed in each petri dish which

plasm. For example, cultivars 157a, 196a, 701a, 728a, and 845a are grown commercially in the United States. Cultivars 196a and 701a represent one extreme type, and cultivars 157a and 728a represent the other extreme type. Cultivar 845a is intermediate in form. If 3 cultivars are to be saved, then one would want to select 196a or 701a, 157a or 728a, and 845a, thus retaining much of the genetic variability of this group. In actual practice, a larger number of cultivars would no doubt be retained for a gene pool and the scatter diagrams could be used to select a diverse group of genotypes.

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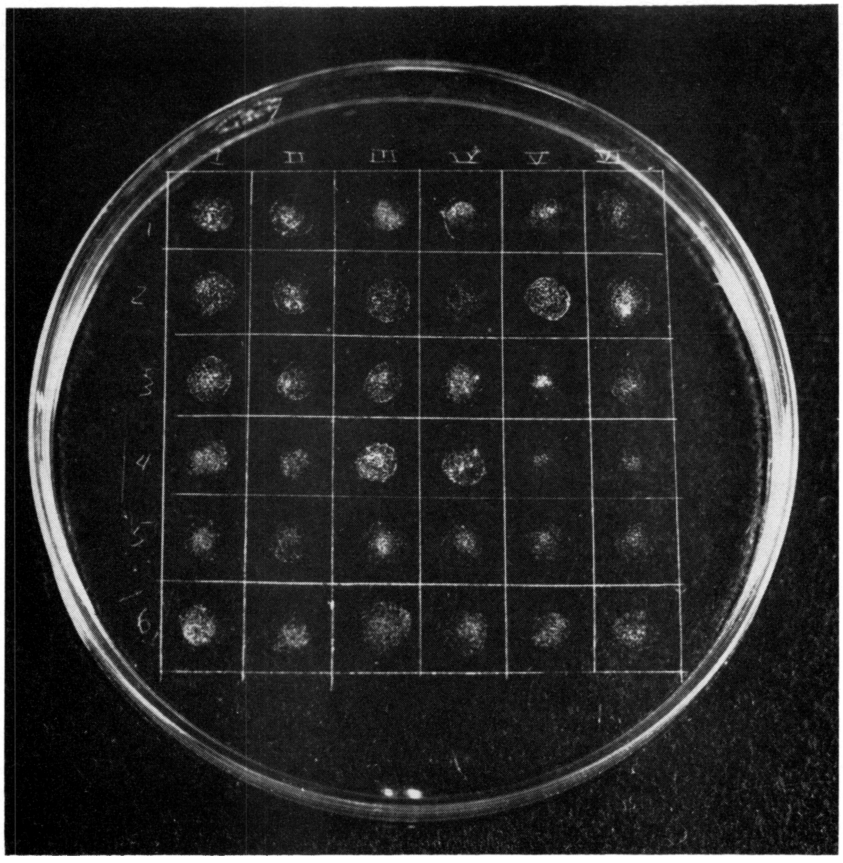

Fig. 1. Petri dish with 36 squares used for hanging drop cultures in pollen germination tests.

was then sealed with a rubber band to maintain a high level of humidity. Since the onion pollen had a tendency to sink, a better dispersion of pollen grains was obtained by allowing the gelatin drops to solidify before the germination dishes were inverted.

Onion bulbs of 2 C-lines, B2215C and B12115C, were grown under greenhouse conditions and were in bloom from approximately June 20 to July 15, 1967. Pollen was selected from flowers of 3 different ages from each of the C-lines. To determine the effects of the time of day on pollen germination, pollen was collected and germinated at 9:00 AM and 2:30 PM. The average greenhouse temperature at the time of collection was 75 and 88° F, respectively. A split-split-split plot design was used in which variety was factor A, time of day was factor B, and age of pollen was factor C.

The age of the pollen was ascertained by morphological changes which occur in the onion flower from the time of anthesis until it shrivels. The onion flower has 2 whorls of stamens with 3 stamens in each whorl. The anthers of the inner whorl of stamens dehisce approximately 1 day before the others. Pollen from these 3 anthers was used for all germinations to keep the ages uniform. Age 1, approximately 1 day after anthesis, consisted of flowers with only 3 anthers dehisced, the stigma very short, the pollen bright yellow, and the petals normal; age 2, approximately 3 days after anthesis, consisted of flowers with all anthers dehisced, the stigma almost as long as the anthers, the pollen bright yellow, and the petals normal; age 3, approximately 6 days after anthesis, consisted of flowers with all anthers dehisced, the stigma fully extended, pollen light tan in color, and the petals slightly shriveled (Fig. 2).

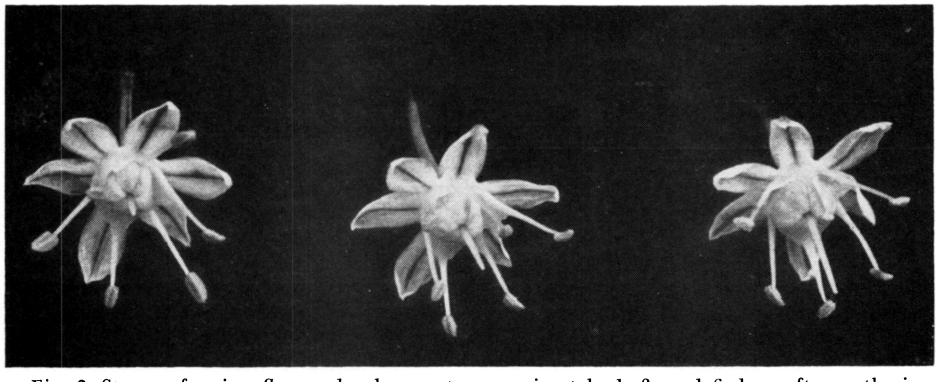

Fig. 2. Stages of onion flower development approximately 1, 3, and 6 days after anthesis showing variation in the number of anthers dehisced, length of stigma, condition of pollen, and degree of shriveling of petals.

Two sets of 3 flowers each of a given age were selected from 3 different umbels. The anthers from the flowers of each set were placed in a spot plate and mixed with the gelatin medium. Two drops of each pollen-gelatin mixture were placed in the appropriate squares of the petri dish. The pollen was allowed to germinate for 1 hr at room temperature (76° F), and photomicrographs were taken of each drop. These photomicrographs were later projected and the percentage of pollen germination for each drop was determined. The percentage was the ratio of the number of pollen grains with tubes at least as long as the diameter of the grain to the total number of grains present.

Onion bulbs of 2 male-sterile lines, B5546A and B2218A, and 2 pollen parents, B2215C and B12115C, were grown under greenhouse conditions and were in full bloom by July 10, 1967. The mid-day greenhouse temperature at this time was approximately 90 to 100° F. Flowers of uniform age from the 2 male-sterile lines were hand pollinated with pollen of 3 different ages from the 2 pollen sources. Five flowers of each parental combination were collected and preserved in a 1:8:1 ratio of formalin, alcohol, and acetic acid (FAA) at 3, 6, and 24 hr after pollination. The styles were later dissected, stained with aniline blue, and observed with ultraviolet light according to a technique by Martin (8). Aniline blue stains the callose, which lines and plugs the pollen tubes and which fluoresces a bright yellow-green under ultraviolet light.

Results and Discussion

Several culture media and techniques were tried to find one suitable for onion pollen germination (Table 1). Onion pollen has a tendency to clump together making a count of individual pollen grains difficult. Better dispersions of pollen grains were ob-

Table 1. The percentage of onion pollen germination on culture media of agar or gelatin with different levels of sucrose, boric acid, and calcium.

Trial	Base medium	Sucrose (%)	H_3BO_3 (ppm)	$Ca(NO_3)_2 \cdot 4H_2O$ (ppm)	Germination (%)
I-plates	1% Agar	5	10	0	25–50 (bursting)
			50	0	25–50
		10	10	0	0
			50	0	70–80
		15	10	0	10–20 (bursting)
			50	0	70–80
II-hanging drops	1% Agar	15	10	0	50–60
			50	0	70–80
	1% Gelatin	15	10	0	50–60
			50	0	70–80
III-hanging drops	1% Gelatin	15	100	0	70–80
				300	100

tained with gelatin in hanging drops than with either agar plates or agar in hanging drops. The optimum levels for sucrose and boron were found to be 10–15% and 50–100 ppm, respectively. The addition of 300 ppm calcium to the germination media resulted in higher percentage germination of onion pollen. These results were similar to those obtained by Kwack (7), cited earlier.

The mean percentages of onion pollen germination as affected by variety, time of day and age are listed in Table 2. None of the interactions were significant; therefore, they were not included in the table. Since the percentages of germination varied widely, the data were transformed into arc-tan $[\tan(\text{arc-sin } X)]^{1/2}$, where X is the original percentage. This transformation was found to normalize these data better than the conventional arc-sin $(X)^{1/2}$.

Results show that the percentage germination of pollen from B12115C was significantly higher at the 5% level than the percentage of pollen germination of B2215C. There was no significant difference between pollen collected and germinated at 9:00 AM and pollen collected and germinated at 2:30 PM. Eršov and Vorob'eva (4) however, reported pollinations in onion at 10:00 AM or 6:00 PM resulted in a higher seed set than pollinations at 2:00 PM. The lower seed set which they obtained may have been caused by a lower humidity at 2:00 PM. In a pollination study such as theirs, humidity could not be controlled while in germination studies humidity was easily controlled.

The percentages of germination of the 3 ages of pollen were significantly different from each other at the 1% level. The ability of onion pollen to germinate declined rapidly after the first day and approached zero by the sixth day after anthesis (Table 2). Probably this decline in ability to germinate would be much faster under field conditions than under greenhouse conditions; thus, the age of pollen is an important factor in onion seed production. The same rapid decline in germination makes other factors such as bee activity, comparative blooming dates of parents, ratio of pollen rows to seed rows, and other cultural practices even more important since the presence of open flowers on the umbels of pollen parents does not necessarily mean this pollen will germinate and result in seed production.

Hand pollination and style studies indicated that the pollen tubes of onion pollen extended from 1/3 to 3/4 of the length of the style in 3 to 6 hr, and the pollen tube grew the entire length of the style, a distance of approximately 4 mm, in 24 hr (Table 3).

Table 2. Mean percentage germination of onion pollen as affected by variety, time of day, and flower age. The data were transformed into arc-tan $[\tan(\text{arc-sin } X)]^{1/2}$ to obtain a normal distribution.

Subclass	Variety	Time	Age[x]	Mean[y]
Variety	B2215C			19.74a
	B12115C			25.90b
Time		AM		21.63a
		PM		24.01a
Age			1	40.80a
			3	23.38b
			6	4.26c

[x]Ages 1, 3, and 6 are defined as 1, 3, and 6 days after anthesis, respectively.

[y]Means within a group accompanied by different letters are significantly different at 5% level. Mean percentage germination values were tested by Duncan's New Multiple Range Test (3).

Table 3. Number and relative length of pollen tubes in the styles of onion flowers 3, 6, and 24 hr after pollination as determined by staining with aniline blue and observation under ultraviolet light.

Relative length	Hours after pollination	Total number observed
33%	3	2
	6	8
	24	0
50%	3	4
	6	5
	24	3
75%	3	5
	6	3
	24	0
100%	3	0
	6	0
	24	7

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