IN VITRO TESTING AND LONG-TERM STORAGE OF BLACK CHERRY POLLEN

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INTRODUCTION

As breeding of black cherry (Prunus serotina Ehrh.) intensifies, there will be an increasing need for effective controlled crossing procedures. Some initial work aimed at this objective has been summarized by Forbes (1973). Pollen handling techniques, including storage and suitable methods for testing viability, are an important part of these procedures. Here we report methods of in vitro germination and the results of storage under several conditions.

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METHODS AND RESULTS

In Vitro Germination.--Fresh pollen used for both the germination studies and the test of storage conditions was collected from flowering branches which had been cut from trees near Norris, Tennessee (300 m. elevation), and placed in water in the greenhouse. Pollen was collected by gently agitating floral racemes over a 40 mesh wire screen.

Pollen from two trees was used in an initial germination test with aqueous and agar media (Table 1). After autoclaving at 15 PSI for 15 minutes, the agar media were plated onto sterile microscope slides; slides with shallow depressions were used for the liquid media. Pollen was dusted on the surface of media and three replications of each tree-medium combination were placed in moist chambers for germination at room temperature. At the end of a 24-hour incubation period, germination percent was determined by counting 100 grains per replicate. Germination was high on all media except water and 10 percent sucrose in water (Table 1). Pollen density on slides ranged from approximately 30 to 150 grainilmm², and there was no apparent relationship between density and germination percent. Due to ease of taking observations and handling, subsequent tests were completed on agar media at a constant 26°C.

Table 1.--Germination percent of fresh black cherry pollen from two trees on various synthetic media.

Germination Medium	Tree A	Tree B
Agar (2%)	97	70
Agar (2%) + sucrose (10%)	97	96
Agar (2%) + sucrose (10%) + boric acid (.01%)	97	73
Water	24	12
Sucrose (10%) in water	30	24
Sucrose (10%) + boric acid (.01%) in water	92	83

In the second trial, freshly collected pollen was also tested on Kwack's (1965) medium, which has been recommended for general use with many horticultural species. Three replications of pollen from each of five trees were used. Results are summarized as follows:

Medium	Mean Germination Percent	Tree-to-Tree Range in Germination Percent
Agar (1%) + Sucrose (10%)	37	17-64
Agar (1%) + Sucrose (10%) + Boric Acid (.01%)	47	32-55
Kwack's Medium (See Table 2 for composition)	49	30-68

Analysis of germination data indicated that germination percent on the agar-sucrose medium was significantly lower than germination on the other two media.

At 26°C, pollen-tube growth was completed in about six hours; final tube length averaged about .4 mm and ranged from .1 to 1.0 mm. depending upon parent tree. No significant variation in the tube length was associated with differences in media.

Storage.--The tolerance of pollen to vacuum-drying and to rapid cooling to low temperatures was determined before long-term storage tests were initiated. Vials of freshly collected pollen were placed on ports of a Virtis Model 10-010 freeze-drier for 15 minutes with and without precooling to -50°C in a dry ice-acetone bath. Prior to testing, vacuum-dried pollen was placed in a recovery chamber (3°C, 50 percent relative humidity) for 12 hours. Other samples were rapidly cooled to -30° and -50°C, by immersion in a dry ice-acetone bath, then thawed rapidly immediately before testing. Four replicates of a pollen mix from several trees were tested on a medium of 1.0 percent agar, 10 percent sucrose and .01 percent boric acid. Average germination percent for individual treatments ranged from 70 to 80, and there were no significant differences among treatments and a control of fresh, untreated pollen.

Storage treatments outlined in Table 3 were subsequently established using pollen from three trees near Norris, Tennessee. Pollen was removed from storage at intervals of 6, 30, and 52 months for germination tests on the medium described above. After 30 months' storage, pollen was also tested on the media outlined in Table 2. Vacuum-dried pollen completely lost viability at room temperature. All other storage treatments retained viability for 30 months. Pollen on the standard sucrose and boric acid medium exhibited slightly but consistently higher germination than pollen on other media, though for most storage treatments there was no practical difference in germination percent due to media. However, only storage at -30°C provided good viability retention to 52 months (Table 3).

CONCLUSIONS

Viable black cherry pollen germinated quickly on a variety of synthetic media, and there appears to be no strict compositional requirement, though sucrose and boron enhanced germination in the aqueous medium. These conclusions are in agreement with the observations

of Klaehn and Neu (1960) who obtained 65 percent germination on 4 percent agar and 10 percent sucrose after pollen has been stored for two weeks at 10°C. A medium of 1 percent agar, 10 percent sucrose, and .01 percent boric acid is provisionally recommended for in vitro testing. Storage in sealed vials at -30°C proved to be a simple, effective way of storing pollen up to four years. For shorter periods, storage in sealed vials at 3°C adequately retains viability, Vacuum-drying appears to be of little advantage unless one requires room temperature storage for several months. As has been frequently observed in other forest tree species, there were major variations in germinability among pollens from individual trees.

Table 2.--Average germination percent of black cherry pollen on various agar media after 30 months' storage.

Storage Treatment		Medium			
Temp., °C	Condition	Sucrose, (10%)	Sucrose, (20%)	Sucrose (10%) Boric Acid (.01%)	Kwack's[a] Medium
3°	Sealed vial	31 (28-36)[b]	64 (56-80)	67 (61-74)	45 (41-49)
3°	25% RH	21 (12-25)	36 (34-40)	30 (25-33)	25 (15-34)
3°	50% RH	33 (15-50)	55 (44-63)	53 (39-62)	46 (30-61)
3°	Vacuum-dried	43 (35-49)	48 (33-65)	54 (46-66)	54 (48-60)
-30°	Sealed vial	80 (75-85)	77 (68-91)	80 (74-85)	69 (62-82)
-30°	Vacuum-dried	76 (64-84)	78 (76-81)	75 (73-77)	61 (45-70)

[a] Sucrose, 10%; calcium nitrate, .03%; potassium nitrate, 01%; magnesium sulfate, .01%; boric acid, .01%.

[b] Range of individual tree means.

Table 3.--Germination of black cherry pollen from trees A, B, and C after three periods under various storage conditions. Germination medium: 1.0% agar, 10% sucrose, .01% boric acid.

Storage Treatment		Storage Time								
		Six Months			Thirty Months			Fifty-Two Months		
Temp., °C	Condition	A	B	C	A	B	C	A	B	C
3°	Sealed vial	88	88	36	67	74	61	31	15	12
3°	25% RH	61	51	57	31	33	25	0	<1	<1
3°	50% RH	68	64	48	62	57	39	24	15	<1
3°	Vacuum-dried	58	71	47	66	49	46	<1	0	<1
-30°	Sealed vial	89	51	66	85	74	82	55	47	47
-30°	Vacuum-dried	81	49	30	73	77	75	28	28	15
21°	Vacuum-dried	71	43	25	0	0	0	--	--	--

LITERATURE CITED

Forbes, D. 1973. Problems and techniques associated with natural and controlled pollination of black cherry (Prunus serotina Ehrh.) Proc. 20th Northeastern Forest Tree Imp. Conf. pp. 47-51.

Klaehn, F. U. and R. L. Neu. 1960. Hardwood pollen study. Silvae Genetica 9(2):44-48.

Kwack, B. H. 1965. The effect of calcium on pollen germination. Proc. Amer. Soc. Hort. Sci. 86:818-823.