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SHORT TERM POLLEN STORAGE OF TWO *Rhododendron simsii* CULTIVARS

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Introduction

Rhododendron breeders work with a genus that collectively has a broad flowering season. However, many individual species or cultivars flower for much shorter periods. To make many desirable crosses, it becomes necessary for the breeder to store pollen. Existing literature offers only brief recommendations on storage conditions in reports by Bowers (1932), Lee (1958), and Schroeder and Bump (1982) and a single, more detailed report by Visser (1955) that few breeders have available.

This study outlines the relative value of a range of storage conditions for short-term (two weeks or less) pollen storage of two cultivars of evergreen azaleas. Long-term storage is generally done successfully by using variations of the desiccator-freezing method, as described by Schroeder and Bump (1982) and Visser (1955).

Materials and Methods

Plants of *Rhododendron simsii* Planch. cultivars, 'Alaska', a Rutherford hybrid, and 'Variegated Dogwood' (Plant Patent 4455), were purchased from a local florist. The plants were about to flower heavily, with many buds just beginning to open.

Anthers were collected from the flowers on 5 January 1986, just as they opened. Two groups of six, unsealed, 2-dram glass vials, with each vial holding three anthers of a single cultivar, were placed in one of six storage conditions. Sample vials were removed from storage at 1, 2, 3, 4, 7, and 10 or 14 days after collection and were allowed one hour to equilibrate to laboratory conditions. Pollen viability was then calculated by using the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazolium bromide (MTT) vital staining meth-

od, as outlined by Hecker (1963), which has been shown to be an accurate indicator of *in vivo* pollen germination in deciduous azaleas (Widrlechner *et al.*, 1983). From each of the three anthers, 200 microspores were scored after staining, making a total sample of 600 microspores per treatment for each cultivar.

As a control, on each day of the experiment, three anthers were sampled from newly opened flowers from the donor plants. Based on these counts, a mean staining proportion was calculated for each cultivar. The test results are then presented graphically with sample staining shown as a proportion of the control means.

The six test conditions for pollen storage were:

Condition A: Anthers were stored at room temperature (17-24°C) with no humidity adjustment (32-42% relative humidity).

Condition B: Anthers were stored at room temperature in a large glass desiccator with anhydrous CaSO₄ as a drying agent.

Condition C: Anthers were stored in an incubator at 20°C for 16 hours per day and 30°C for 8 hours per day with 80-100% relative humidity.

Condition D: Anthers were stored in a refrigerator at 4°C.

Condition E: Anthers were stored in a freezer at -18°C.

Condition F: Anthers were first placed in a large glass desiccator, as in Condition B, for 18 hours; then they were moved for storage in the freezer along with samples of Condition E.

Results and Discussion

Based on a total of 4200 microspores compiled from measurements taken on seven days, the

mean proportion of microspores of 'Alaska' stained by MTT is $.356 \pm .078$ (± 1 S.D.). For 'Variegated Dogwood', the proportion is $.408 \pm .058$. These figures are considerably lower than found in the majority of deciduous azalea cultivars, as surveyed by Widrlechner and Pellett (1983), but are consistent enough within each cultivar to use for this experiment. To date, no comparable survey of pollen viability of evergreen azalea cultivars has been published.

The results of the room temperature storage conditions, Conditions A, B, and C, are presented in Figure 1. Figure 1 contrasts days in storage with staining, as expressed as a proportion of the mean of the control samples for both cultivars. The letters indicate the mean values of each condition on a given day for the two cultivars, and the dotted lines indicate the proportion of staining found at ± 1 S.D. from the control mean. If storage treatments did not affect pollen viability, approximately two-thirds of the data points would fall within the area demarcated by the dotted lines. This was not the case.

Condition A, ambient room storage, gave good results, with staining over 80% of control levels up to four days after collection. Anthers were easy to manipulate, but became rather brittle by Day 10.

Condition B, desiccator storage, also gave good results, with staining over 80% of control levels up to four days. However, by Day 4, the anthers had dried to a state that made them very difficult to handle. On Day 10, the anthers would shatter when picked up with forceps. Storage in a desiccator with CaSO₄ past four days cannot be recommended.

Condition C, high-humidity storage, was much less successful. The

proportion of pollen staining was consistently lower than Conditions A or B, and the anthers deteriorated visibly while in storage. By Day 7, the texture of the anthers became quite flaccid and on Day 10, the anthers were all discolored and soft. *Rhododendron* pollen should not be stored under high-humidity conditions. This finding is in agreement with Visser's (1955) results using *R. catawbiense* Michx. and *R. molle* (Blume) G. Don. High humidity is also detrimental to the survival of the pollen of pearl millet (Sarr *et al.*, 1983), grapes (Randhawa *et al.*, 1982), and many other species (Visser, 1955). There are some exceptions to this generalization (see Stanley and Linskens, 1974).

Analagous to Figure 1, Figure 2 shows the results of the cold-storage tests, Conditions D, E, and F.

Condition D, refrigerated storage, gave acceptable results, with treatments varying between 69% and 91% of the control means. No deterioration in anther quality was observed. These results are in contrast to the advice of Bowers (1932), who stated that refrigerated storage was poorly suited for *R. catawbiense* pollen.

Condition E, freezer storage with no desiccation, gave unacceptable results. In four of 36 anthers sampled, no viable pollen was found. Extremely low pollen viability was found in all samples of the 'Alaska' cultivar. 'Alaska' has anthers that appear more moist than those of 'Variegated Dogwood' shortly before and at anthesis. It is probable that high moisture content at freezing causes irreversible damage to the pollen (Stanley and Linskens, 1974; Visser, 1955). Direct freezing cannot be recommended.

Condition F, freezer storage following desiccation, generally gave the best results of all storage conditions (five of six test dates). Schroeder and Bump (1982) and Visser (1955) agree that desiccated, frozen *Rhododendron* pollen will remain viable for at least two years. This study confirms their observations on the necessity of desiccation and recommends this storage method for short-term storage as well. At room temperature, 18 hours under desiccating conditions were sufficient to dry

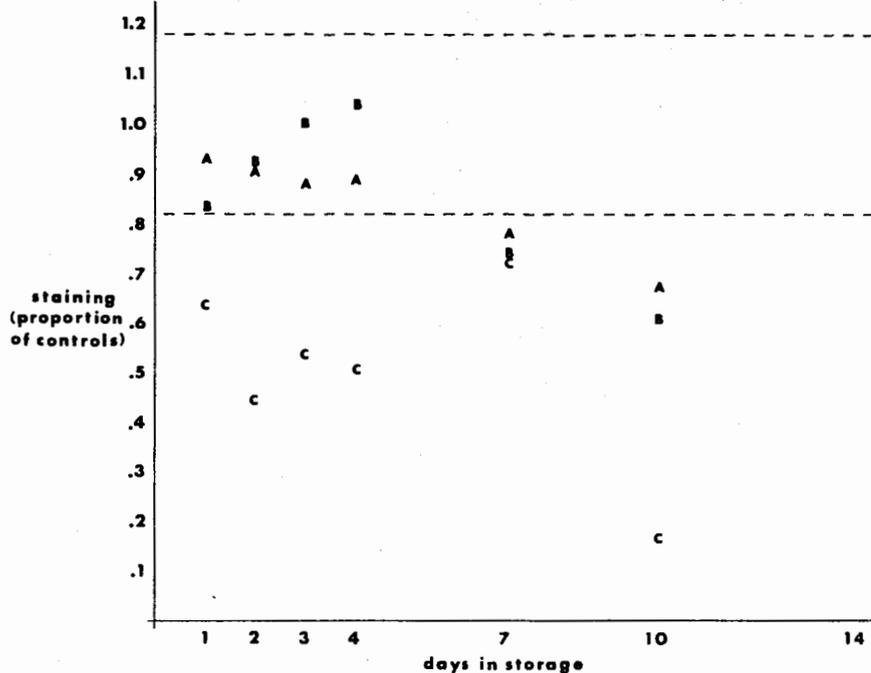


Figure 1. The relationship between pollen storage time and proportion staining for Conditions A (room temperature), B (desiccator storage), and C (high-humidity storage).

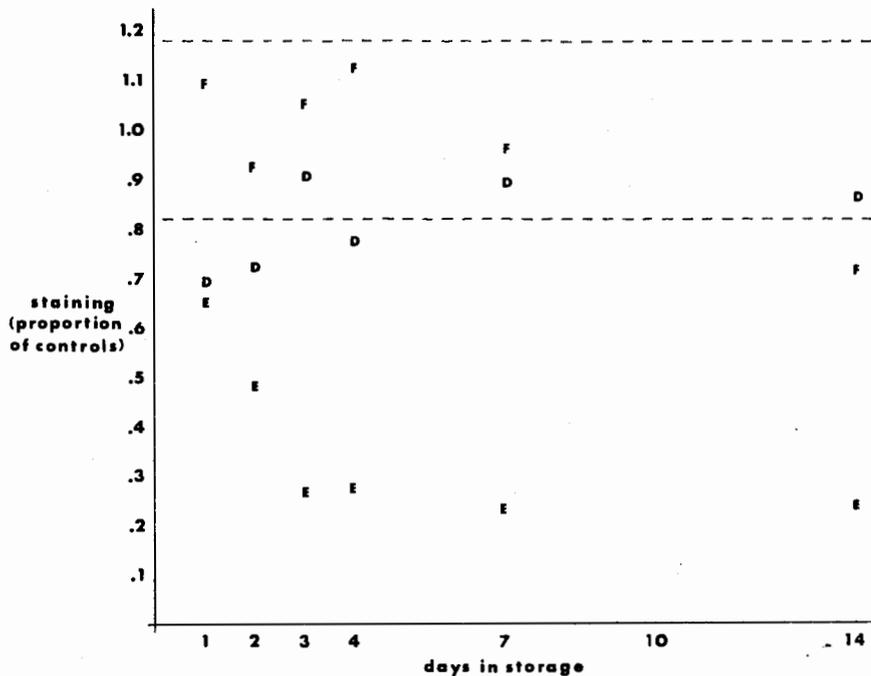


Figure 2. The relationship between storage time and proportion staining for Conditions D (refrigerated storage), E (freezer with no desiccation), and F (freezer following desiccation).

the pollen, as compared with the three to seven days of refrigerated desiccation suggested by Schroeder and Bump (1982).

Conclusion

Six storage methods for holding evergreen azalea pollen up to two weeks were compared. The best method of those tested is storage of samples in a freezer following 18 hours in a desiccator at room temperature. Ambient room conditions could be used for storage of up to four days, but humidity needs to be kept low. Desiccator storage and refrigerated storage gave intermediate results. Whereas high-humidity storage and freezer storage without prior desiccation proved unacceptable.

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Mark Widrechner, horticulturist with the USDA-Agricultural Research Service, Ames, Iowa, currently serves on the ARS Research Committee and as a technical advisor to the ARS Journal.

BRONZE MEDAL AWARDS

CONNECTICUT CHAPTER

Lorenzo F. Kinney, Jr.

Known as "The Azaleaman", Lorenzo F. Kinney, Jr., began collecting azaleas with the idea of testing their suitability for growing in the Kingston, R.I. area; this, during a time when azaleas were grown in the north mainly as greenhouse plants.

When B.Y. Morrison first introduced his new Glenn Dale hybrids, Mr. Kinney saw this as a challenge and a possible opportunity to bring new color and diversity of form to northern gardens. Over the years, he has tested and grown most of the Glenn Dales. He visited Joseph Gable frequently and now has most of his hybrids. More recently, he has added Gartrell, Linwood, North Tisbury, and other hybrids to his plantings. All of these, including some of his own creations, are presented in all their glory to azalea lovers who flock to the "Azalea Tea"

given by Mr. and Mrs. Kinney each year.

Mr. Kinney has been a member of the Connecticut Chapter for nineteen years. He drives the long distance to our meetings in fair weather and foul. Our show tables have been graced by his beautiful entries, and we have enjoyed his interesting and informative comments.

His boundless enthusiasm for azaleas has encouraged the uninitiated to expand their horizons beyond the common clones of yesteryear. Let no one say that azaleas are poor relations of rhododendrons!

Presentation of the Bronze Medal of the American Rhododendron Society gives us an opportunity not only to praise one of our loyal members, but also to acknowledge our gratitude and respect for him. Tonight the Rhododendron Society of Connecticut is pleased to honor Lorenzo F. Kinney, Jr.

April 11, 1986.

PORTLAND CHAPTER

Betty Spady

In appreciation of her many active years of membership in the American Rhododendron Society as a life member of the Portland Chapter and a member of other chapters. For being active in forming the Western Regional Fall Conference. For taking care of many details, making each Western Conference a success. For serving on committees, presently the Membership Committee. For being Chairman of the Roster Committee. For her graciousness and friendliness to everyone. This Chapter is pleased to give its highest honor and recognition, the Bronze Medal, to Betty Spady by unanimous decision of the Honors Committee, Portland, Oregon — May 1986.