Special Research Report: #517: Production Technology

Assessing the Pollen Viability of *Clerodendrum*

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BACKGROUND

The genus *Clerodendrum* with over 400 species has a wide variability in characteristics such as inflorescence shape and color, leaf shape, and plant form. These characteristics make *Clerodendrum* spp. candidates for container production and use as flowering potted plants. Interspecific hybridization of these species could further the use of this genus for container production. Unfortunately, there is limited information published on *Clerodendrum* hybridization. Determining pollen viability is an important initial steps in establishing a breeding program. Preliminary experiments indicated that interspecific crosses of various *Clerodendrum* species produced no seed set. The object of this experiment was to determine the duration of pollen viability after anthesis in *C. speciosissimum* and *C. thomsoniae*, two promising species.

MATERIALS & METHODS

Cellular stains are commonly used in plant breeding to assess pollen viability in flowering plants. The MTT (2,5 – diphenyl tetrazolium bromide) test was selected as the best method for this study. Pollen was collected every day from anthesis to flower abortion from *C. speciosissimum* and *C. thomsoniae* grown in a greenhouse. Flowers (Figures 1 and 2) were tagged the day of flower opening. Anthers from the opened flowers were removed and placed into scintillation vials with the tags. Each day, the vials were placed into a dehiscer for approximately one hour before transferring the pollen to slides. Using a micropipette, approximately 0.05mL of MTT media was transferred to 1.5mm microscope slides that had been sectioned into quadrants using a permanent marker. Three slides were used per species per day. The slides were allowed to cool to room temperature (~ 72^{o}F) and pollen was transferred onto the media using a fine artists brush. Percent viability was determined after one hour with an *Olympus BH-2* light microscope at x100 magnification by counting the number of stained pollen grains. *C. speciosissimum* was found to reach stage IV (flower abortion) after 5 days; while, *C. thomsoniae* reached stage IV in only 4 days. Therefore, the viability data for *C. speciosissimum* was collected over a 5-day period and for *C. thomsoniae* over a 4-day period.

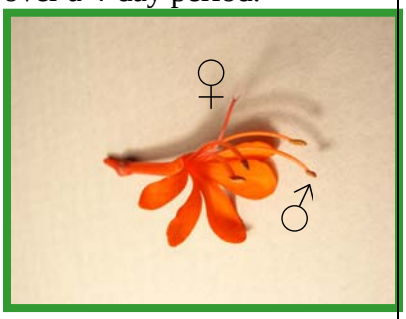

Figure 1. Flower of *C. speciosissimum*.

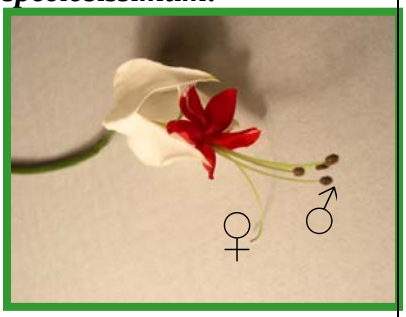

Figure 2. Flower of *C. thomsoniae*.

RESULTS

The results showed a correlation between flower

age and percent viable pollen. There was no difference for both species between DAF 1 and 2 (DAF= days after flowering) that pollen was collected. The highest percent viable pollen was attained the first two days after flowering. In *C. thomsoniae*, the first two days after anthesis that pollen was collected there was only a decrease of 5.46% from DAF 1 – DAF 2 occurred. The greatest decrease occurred between DAF 2 and 3 with a decrease in viability of 35.66%. This large decrease, however, was immediately followed by an equally large increase in viability (33.26%) between DAF 3 and 4. In *C. speciosissimum* the results showed a similar pattern, but there were two key differences. The first two days showed the greatest percent viable pollen with DAF 1 at 74.53% and DAF 2 at 60.598%. The percent change between DAF 1 and 2 for *C. speciosissimum* was markedly greater with a decrease of 18.69%. Between DAF 2 and 3, the same trend was found as in *C. thomsoniae*, with a decrease in pollen viability of 22.12%. From DAF 3 to DAF 5 the percent viability remained relatively constant at approximately 50%.

Scanning electron micrographs of pollen grains for both *Clerodendrum* species (Figures 3, 4, 5, and 6) decrease in number and shape and they became more distorted as the DAF increased.

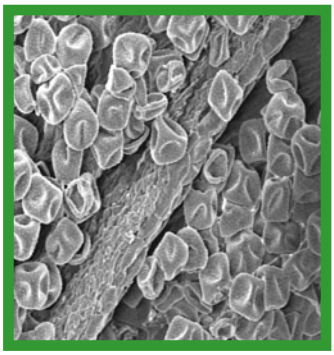

Figure 3. *C. speciosissimum* pollen grains at DAF 1.

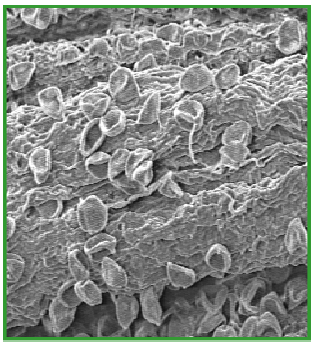

Figure 4. *C. speciosissimum* pollen grains at DAF 3.

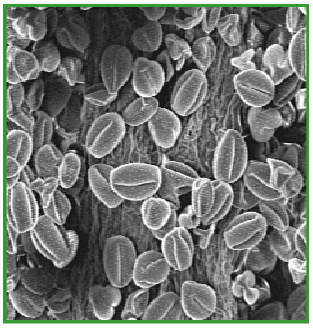

Figure 5. *C. thomsoniae* pollen grains at DAF 3.

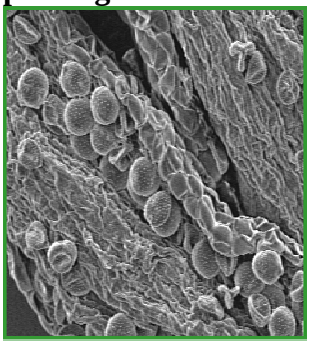

Figure 6. *C. thomsoniae* pollen grains at DAF 1.

CONCLUSIONS

Pollen viablitiy *C. speciosissimum* and *C. thomsoniae* was highest for the first 2 days after anthesis. The larger fluctuations in viability values for *C. thomsoniae* are possibly an indirect result of the behavior of the anthers. The tight coiling of the anthers on DAF 2 caused problems with sample collection and may be responsible for the loss of the pollen still attached to the anthers. A reason for the apparent increase in viability of the third day is most likely due to testing error. If pollen is collected up to 4 days after flowering, viability was 50% higher. This study is of value for *Clerodendrum* breeding because of the lack of established techniques for crossing. Future research is needed to determine the time of greatest stigma receptivity to viable pollen.

IMPACT TO THE INDUSTRY

None at this time.

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