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A Pollen Chase Experiment; Examining Varying Levels of Embryonic Inbreeding Depression

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Abstract

- A pollen chase experiment was performed upon three Costa Rican populations of *Witheringia solanacea* to examine the breakdown of genetically enforced self incompatibility (SI) and the extent of embryonic inbreeding depression.
- Self-pollen was applied in the bud, with outcross pollen applied one day later, and outcross pollinations at both intervals as a control.
- A variety of responses were found among the populations
 - BOHS readily accepted self pollen and suffered from very low inbreeding depression.
 - Monteverde and Las Cruces both have lower fruit set with self-pollination precedence indicating that bud pollinations can overcome the self-incompatibility response and that embryonic death due to inbreeding depression causes fruit failure.
- The treatment:control fruit set is higher for the Las Cruces plants indicating stronger SI response
- Self-precidence seeds from the Las Cruces plants are likely to be outcrossed
- Self-precidence seeds from Monteverde are likely selfed



Materials and Methods

- Performed bud pollinations on greenhouse *Witheringia solanacea* plants from three locations in Costa Rica: Monteverde, Las Cruces and BOHS.
- Treatment: force almost fully developed bud open, self pollinate with mature anther, outcross 24 hours later when mature (Fig. 1)
- Control: force almost fully developed bud open, outcross with mature anther, outcross 24 hours later when mature (Fig. 1)
- Monitored the status of flowers while maturing, noted if aborted or became fruit
- Harvested any fully developed fruits of self and outcrossed flowers and counted seeds
- Performed non-parametric t-tests on fruit set and seed number for the three populations

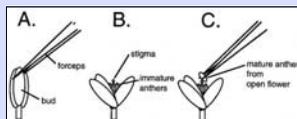


Figure 1: For pollen chase experiment, open an almost fully mature bud with forceps (A), locate the stigma (B), and pollinate with a mature anther from an open flower

Results

- BOHS--no significant difference between self-precidence (treatment) and outcross (control) fruit sets (non-parametric t-test, N=3, $z=0.22$, $p>0.05$, Fig. 2), and no significant difference between self and outcross seed numbers (non-parametric t-test, N=2, $z=1.5$, $p>0.05$, Fig. 2)
- Monteverde--significantly lower treatment fruit set than the control (non-parametric t-test, N=18, $z=2.67$, $p=0.007$, Fig. 2), significantly lower treatment seed number than control (non-parametric t-test, N=8, 14, $z=2.66$, $p=0.008$, Fig. 2)
- Las Cruces--significantly lower treatment fruit set than control (non-parametric t-test, N=11, $z=2.20$, $p=0.03$, Fig. 2), no significant difference between treatment and control seed number (non-parametric t-test, N=4,8, $z=0.34$, $p>0.05$, Fig. 2)

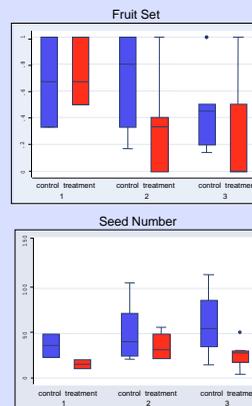


Figure 2: Fruit set and seed number for BOHS (1), Las Cruces (2), and Monteverde (3) control and treatment flowers.

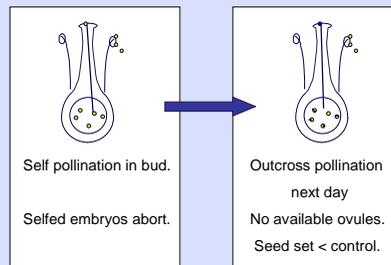


Figure 4: Predicted for plants with strong embryonic inbreeding depression, but bud pollinations overcome SI, allowing self pollen tubes to grow (Monteverde)

Conclusion and Discussion

- BOHS plants are selfing with no observable inbreeding depression
- Las Cruces and Monteverde both have a lower fruit set for the treatment, suggesting that the bud pollinations are successful at overcoming the self-incompatibility response (Fig. 3), and that inbreeding depression is leading to embryonic death, causing failure of the fruit set
- Las Cruces plants have a higher ratio of treatment:control fruit set, which shows that they have a stronger SI response
 - Supported by pollen tube and RNase data (Stone et al, 2006)
 - Predict seeds from Las Cruces will be outcrossed due to the death of selfed seeds from a combination of stronger SI and severe embryonic inbreeding depression (Fig. 4)
- Monteverde self precedence seeds may be selfed and not outcrossed due to a weaker SI response (Fig. 5)
 - More pollen tubes reach base of the style, lower stylar RNase activity (Stone et al, 2006)
 - Two plants from Monteverde (MV24 and MV2) are strong selfers with little inbreeding depression and inflated the Monteverde ratio
- Genotyping the surviving progeny will show whether more self seeds do survive in the Monteverde population than for Las Cruces

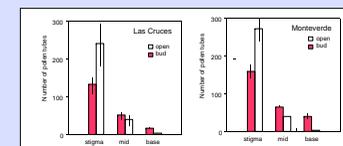


Figure 3: Bud pollinations permit pollen tubes to overcome SI (Stone et al, 2006).

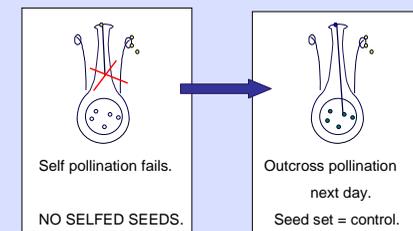


Figure 5: Predicted for plants with strong embryonic inbreeding depression, whose bud pollinations do not completely overcome SI, killing self pollen tubes and allowing outcross pollen to reach ovules (Las Cruces).