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Wilson, Emily and Stone, Judy, "A Pollen Chase Experiment; Examining Varying Levels of Embryonic Inbreeding Depression" (2007). *Undergraduate Research Symposium (UGRS)*. 48.
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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

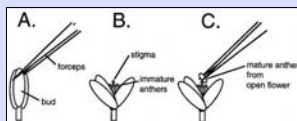


Figure 1: For pollen chase experiment, open an almost fully mature bud with forceps (A), locate the stigma (B), and pollinate with a mature anther from an open flower

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)

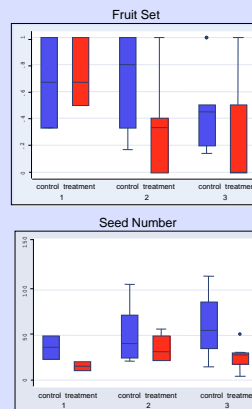


Figure 2: Fruit set and seed number for BOHS (1), Las Cruces (2), and Monteverde (3) control and treatment flowers.

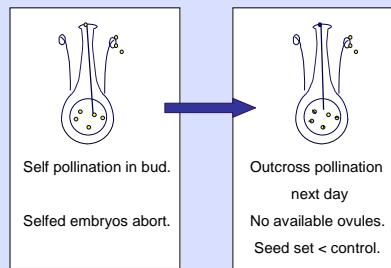


Figure 4: Predicted for plants with strong embryonic inbreeding depression, but bud pollinations overcome SI, allowing self pollen tubes to grow (Monteverde)

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

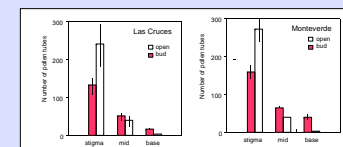


Figure 3: Bud pollinations permit pollen tubes to overcome SI (Stone et al, 2006).

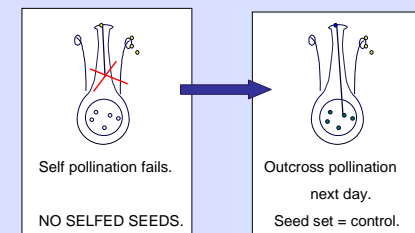


Figure 5: Predicted for plants with strong embryonic inbreeding depression, whose bud pollinations do not completely overcome SI, killing self pollen tubes and allowing outcross pollen to reach ovules (Las Cruces).

Works Cited
Stone, Judy L., Sasuclark, Miruna A., Blomberg, Chris P. 2006. Variation in the self-incompatibility response within and among populations of the tropical shrub *Witheringia solanacea* (Solanaceae). *American Journal of Biology*. 93: 592-598.