A thirst for more:

Morphological changes in *Arceuthobium americanum* indicate water theft from its host, *Pinus contorta*

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Introduction

- Dwarf mistletoes (DMs) are a group of dioecious parasitic flowering plants[1]
- *Arceuthobium americanum* (lodgepole pine DM) infects *Pinus contorta* (lodgepole pine) (Fig. 1) and stunts the host's growth
- DM infection increases the host's vulnerability to abiotic stresses (such as drought)[1]
- *A. americanum* invades ray parenchyma of the tree and consequently steals the host's water[1]
- *A. americanum* explosively-discharges its seeds
- This process is dependent on a buildup of hydrostatic pressure within the fruit
- **Objective:** As stomata are involved in water conductance, we aimed to gain a greater understanding of their role in DM pathology

Methods

Female DM shoots from the same five trees were collected once per week from April-August 2015 from Stake Lake, BC

Second-year fruits were mounted on aluminum stubs and visualized using environmental scanning electron microscopy (eSEM)

Micrographs were analyzed with ImageJ to determine stomatal density and fruit volume; Polynomial regression was used to reveal trends

Figure 1. Female *A. americanum* parasitizing *Pinus contorta*. The aerial shoots erupt from the bark after 4-5 years of incubation within the host's wood. Flowers are pollinated in their first year, and fruits explosively discharge their seeds the following year. Bar: 2 cm

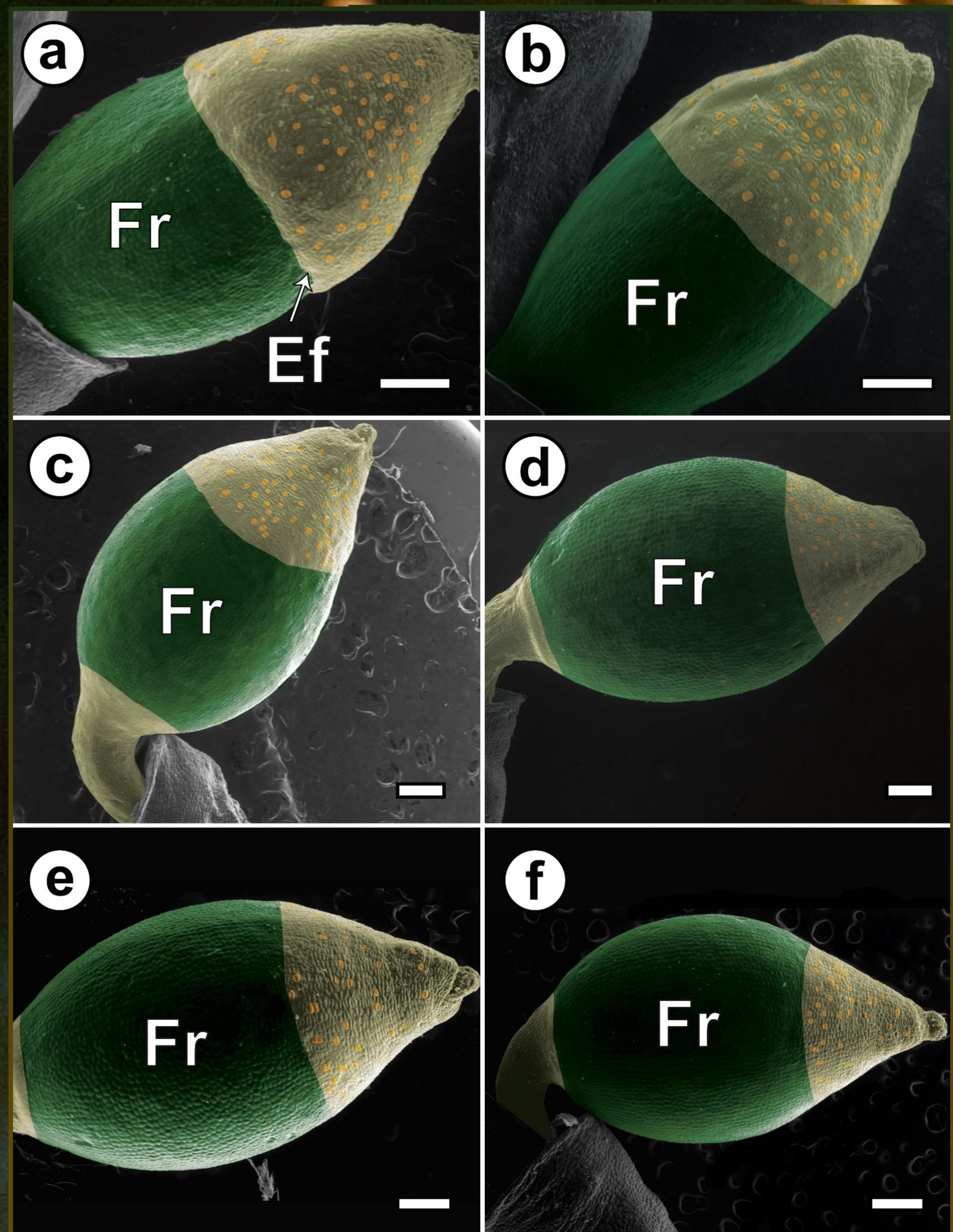

Figure 2. Second year DM fruit eSEM images taken in 2015 on **(a)** April 29 **(b)** May 28 **(c)** June 30 **(d)** July 28 **(e)** August 25 and **(f)** September 1. The fruit body (Fr) is shown in green, sepals in beige, and subsidiary cells of each stoma in orange, An epidermal fold occurs (Ef) where the sepals are inserted at the ovary. Visually, development is expressed by an expansion in diameter and an increase in length with a declining stomatal density and loss of the Ef by May. Floral organs persist through the growing season. Bar: 400 µm.

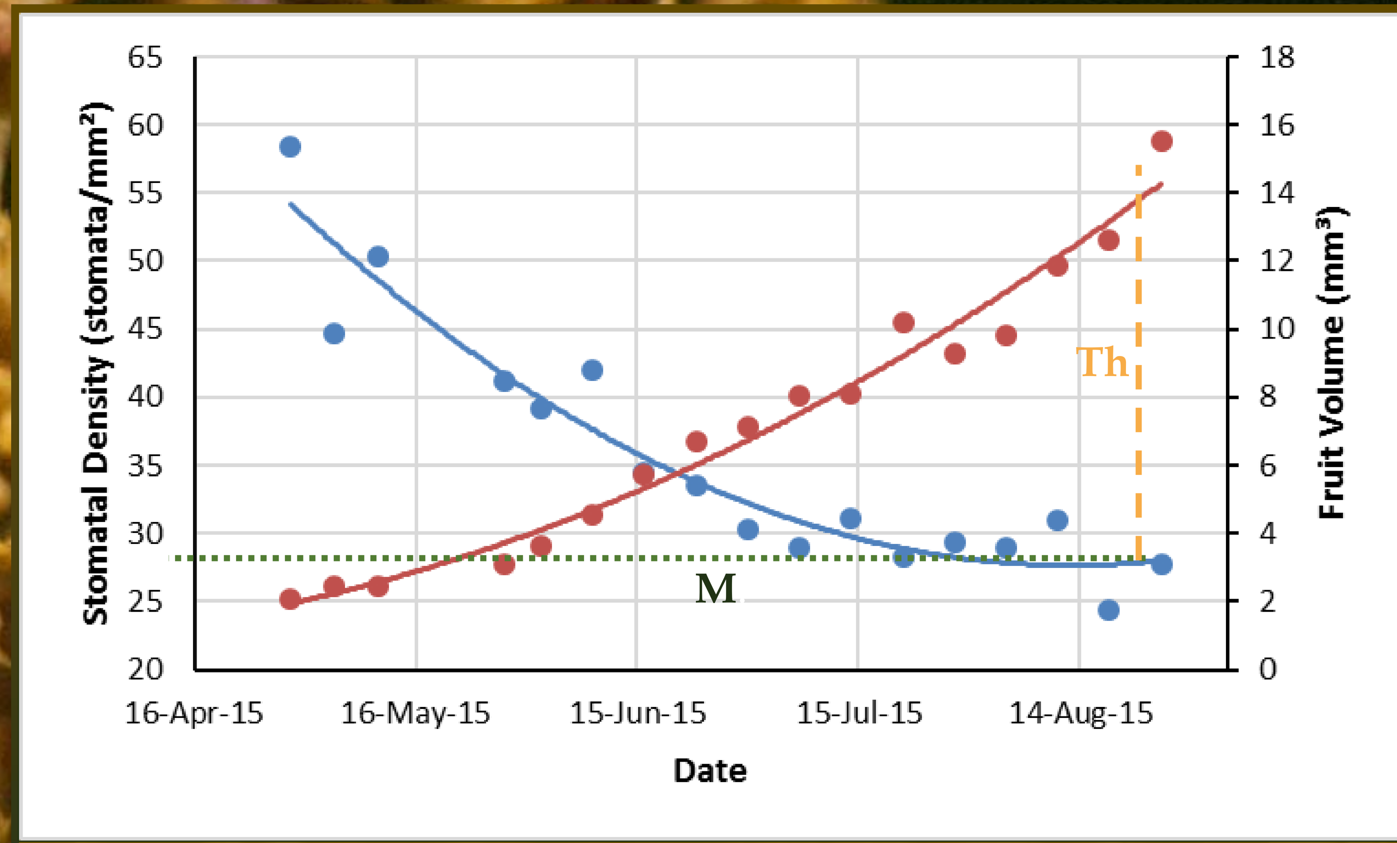

Figure 3. Stomatal density (blue) and fruit volume (red) relationship over time in second-year DM fruits. The stomatal density levelled at the maintenance point (M), at 27 stomata/mm². The difference between density and volume when discharge occurs is the threshold (Th).

Results & Discussion

- Stomata were found only on the flowers, stems, and reduced leaves, never on the fruit body (Fig. 2)
- Stomatal density ($p<0.001$) and fruit volume ($p<0.001$) changed significantly through the season (Fig. 3)
- Initial floral stomatal density (55 stomata/mm²) is greater than the abaxial surface of the leaves (38 stomata/mm²)[1]
- Flowers may provide water conducting force for the DM, analogous to leaves of other angiosperms
- *A. americanum* uses thermogenesis for seed dispersal[3] and requires exogenous heat[2]
- We propose stomatal density declines to the minimum point required for homeostasis after which the difference between stomatal density and volume reaches a threshold, inducing discharge through lack of transpiration-driven cooling (activating thermogenesis) and by excessive accumulation of hydrostatic pressure

References

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3. deBruyn, R.A.J., Paetkau, M., Ross, K.A., Godfrey, D.V., and Ross Friedman, C. 2015. Thermogenesis-triggered seed dispersal in dwarf mistletoe. Nat. Commun. **6**: 6262.

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