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DIAGNOSING AVOCADO POLLEN ABNORMALITIES BY USING A NEW NOVEL COUNTING METHODOLOGY

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ABSTRACT
A new method for pollen counting in avocados needed to be developed as it is impossible to consistently cut anther samples at identical depths for light microscopy investigation. This new pollen-counting methodology therefore addresses inconsistent cutting depths in semi-thin sectioning preparations for light microscopy. This method effectively distinguishes between 'healthy', 'deformed', and 'empty' pollen in avocado (*Persea americana* Mill.) anthers, offering insights into pollen development under cold stress as preliminary findings suggest that proper pollen maturation in avocados is temperature-dependent, particularly during late flower development.

INTRODUCTION
Cold stress adversely affects avocado flowering, causing unsynchronized blooming and low yields. Avocados rely heavily on insect pollinators, and their flower structure act as a barrier to self-pollination (Radar *et al.,* 2020). Given the low pollination rates and the impact of cold temperatures on pollen viability, this study employed a novel pollen-counting method to assess pollen health in two *P. americana* cultivars, using light microscopy.

MATERIALS AND METHODS
The materials and methods of this novel counting methodology are outlined and described in Stanton and Du Toit (2022), but are summarised as follows:

Flower Collection and Anther Fixation:
Samples were taken from 12-year-old avocado trees ('Hass' and 'Fuerte') at the University of Pretoria. Flowers were collected on warmer and cooler days, and anthers were fixed in a glutaraldehyde solution.

Microscope Preparations:
Anthers were dehydrated in ethanol series and embedded in epoxy resin. Semi-thin sections were stained with Toluidine blue for analysis.

Pollen Counting Methodology:
Pollen counts were normalised to compartment size, calculated by multiplying compartment length and width (see Figure 1 in Stanton and Du Toit, 2022).

RESULTS AND DISCUSSION
Anthers showed variability in pollen content, with some having missing compartments (see Figure 2 in Stanton and Du Toit, 2022).

Pollen was categorized as 'healthy', 'deformed', or 'empty' (see Figure 3 in Stanton and Du Toit, 2022).

According to Stanton and Du Toit (2022), 'Fuerte' anthers had more 'empty' pollen, while 'Hass' showed more 'deformed' pollen, particularly under cooler conditions. This suggests that cold temperatures influence pollen development differently across cultivars, and that 'Fuerte' flowers appear to be able to produce more pollen under cooler conditions while 'Hass' flowers produce more pollen under warmer conditions.

CONCLUSION
This novel pollen counting methodology provides a reliable way to assess pollen health in avocados. The study also indicates that cold temperatures influence pollen development, with potential implications for improving pollination and fruit set in avocados. The methodology was therefore applied and validated in a SAAGA project during 2022/23, with contributions from Elsa S. du Toit (project leader, University of Pretoria), Michelle Stanton and Demi Tait (MSc students), Arthur Sippel (project coordinator, ARC-TSC, Tzaneen), Andani Mmboyi (researcher, Tzaneen), and Salomie Willemse (researcher, Stellenbosch).

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