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Title: **Developing new pollen grain count method using machine learning to evaluate heat tolerance of tomatoes**

For fruit and seed bearing plants, pollination is essential for crop production. Pollen production and viability is extremely sensitive to heat stress. Developing reliable and efficient method for counting pollen grain is needed for screening pollen heat-tolerance. In viable pollen grains, fluorescein diacetate (FDA) is converted to fluorescein by cytoplasmic esterase activity and propidium iodide (PI) labels pectin in cell walls. Dead pollen grains are characterized by an absence of esterase activity and thus lack fluorescein-derived fluorescence and become permeable to PI, therefore displaying PI fluorescence inside the pollen grain. Images acquired under a fluorescent microscope have viable pollen in green and dead pollen in red. The Image J software application is the most popular tool for analyzing images and count pollen grains in image samples. However, uneven distribution of pollen grains on glass slides has been a major challenge for the reproducibility of Image J driven results. Through the collaboration between TSU Ag and Computer Science, we have developed a new method for automatic counting of viable and dead pollen grains from microscopic imaging.

In this project, tomato plants were exposed to high and mid-heat stress conditions. Upon release of tomato pollen, samples were stained with a combination of FDA and PI. A 5-10 ml of pollen suspension was loaded onto glass slides and imaged under a ZEISS microscope. A novel computer vision framework is developed for fully automatic processing and analysis of raw microscopic image tiles to detect and count the presence of viable and dead pollen. The framework applies a sequence of morphological techniques to the input image to first detect and then count the viable pollen by rendering contour across each pollen with an identification number. Upon counting, the viable pollen grains are masked to facilitate unambiguous detection and counting of dead pollen in the second phase. Our qualitative and visual assessment of pollen contours reveals almost perfect count for the viable pollen and the accuracy of dead pollen count within a minor error margin. The framework under development is expected to improve in the future through the application of advanced image augmentation and deep learning methods. Using this method, pollen heat tolerance in different tomato varieties is evaluated. The project activities are conducted by students in Agriculture and Computer Sciences This project is funded by a USDA-NIFA-CBG grant.