

## New and rapid method for producing stable appressoria of the genus *Colletotrichum*

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**Purpose:** Morphological features of appressoria is one of the most important criteria for species level classification of the genus *Colletotrichum*. Currently, the common method to study appressoria in *Colletotrichum* is to grow the fungus on SNA media and observe appressoria through the underside of the petri dish. However, this method does not provide stability in appressorial shape, and it could take more than a month for some appressoria to form, and sometimes no appressoria are found. To create a stable and fast method to obtain appressoria.

**Methods:** Six different extraction methods from tomato peels were evaluated by the following procedures: 5 µl of the extraction fluid was placed on a sterilized slide glass and dried, and then 20 µl of a conidial suspension ( $5 \times 10^4$  conidia/ml) were placed on this slide and incubated for 12 to 16 hours at 25 C. For comparison of the previous method and our new methods, appressoria of five strains belonging to different species complexes (*C. higginsianum*, *C. gloeosporioides*, *C. orbiculare*, *C. spaethianum*, and *C. truncatum*) were evaluated.

**Results:** The results showed that extraction fluid by 80% ethanol (50 ml) from 2 g tomato peels overnight was the most effective for optimal appressoria number and size. The standard deviations (n = 30) of the length and width of appressoria using the new method (0.6 to 1.2 and 0.4 to 0.7, respectively), were significantly less than on SNA media (2.2 to 3.1 and 0.8 to 1.4).