

## **Appendix A. Description and operation of the Kotzé Inoculum Monitor**

The Kotzé Inoculum Monitor (KIM) (Fig. A.1) operates through an electric evacuator motor, which requires a power source of 220/250 volt AC. The evacuator motor extracts air from the low-pressure chamber, resulting in the air being drawn via the plant material contained in a circular plastic grid into the funnel-shaped hopper. Velocity of the spore-laden air increases considerably as it is sucked through the gradually tapering ducting and eventually a jet of air passes through the orifice at the base of the ducting. At a distance of 3 mm away from the exit of the orifice, and in a plane perpendicular to the jet of accelerated air, the air strikes the surface of an adhesive-coated standard microscope slide or a Petri dish (65 mm diam.) containing nutrient medium, causing the spores to adhere to the sticky surface.

The microscope slide or Petri dish is mounted on a carrier frame (Fig. A.2), which, over a period of two hours, moves linearly through a distance of 30 mm. This movement is achieved by means of a clockwork device and switch combination. The purpose of this movement is to deposit spores over an extended area that could be related to the actual time of spore release.

Winding of the clockwork automatically switches on the evacuator motor and starts the two-hour operating cycle. On completion of the cycle, the evacuator motor switches off as the clockwork fully unwinds with an audible click. The construction of the air-duct, which connects the hopper to the low-pressure chamber, is designed so that water dripping from the plant material is drained away from the system without affecting the functioning thereof. The complete stainless steel construction of the KIM protects the operating process from the effects of static electricity.

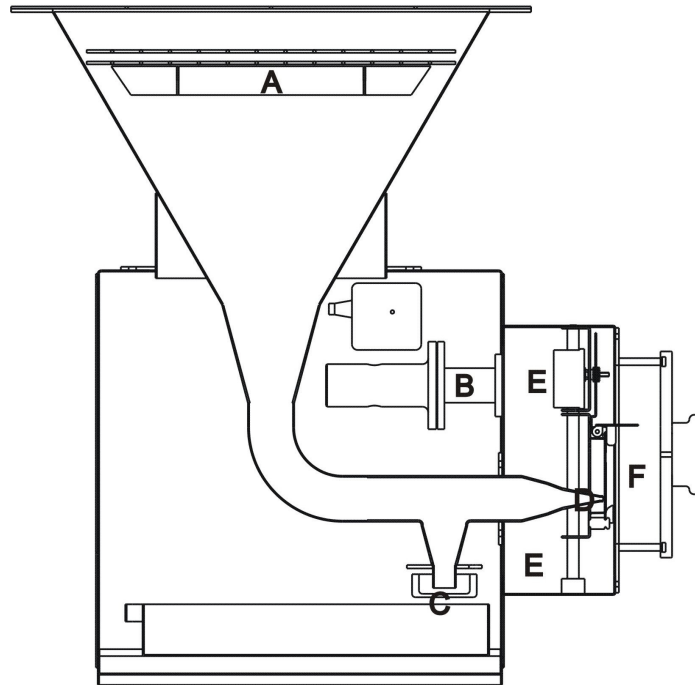


Figure A.1. Diagrammatic cross-section of the Kotzé Inoculum Monitor unit. A: grid support for plant material in hopper; B: evacuator motor unit; C: water trap with overflow water tray; D: air orifice; E: low-pressure chamber; F: carrier for Petri dish or microscope slide.

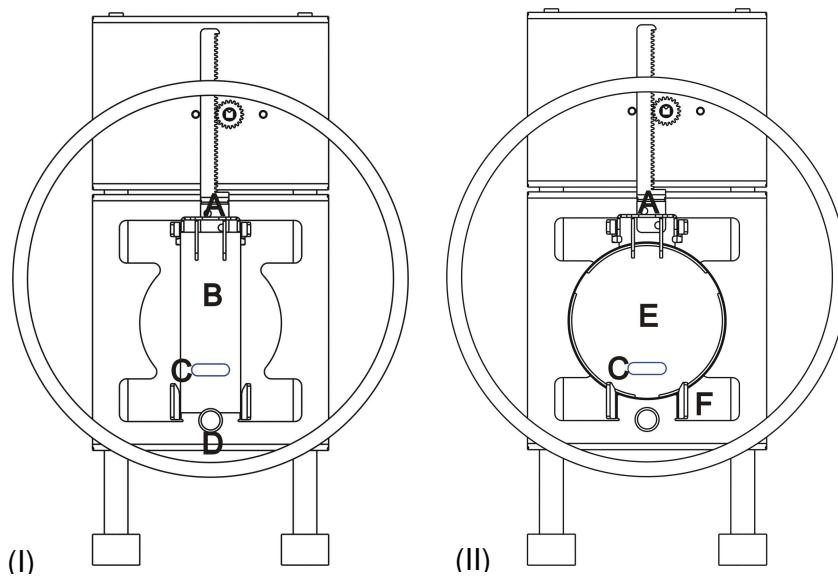


Figure A.2. Diagrammatic cross-section of the front view of the low-pressure chamber to illustrate position of microscope slide (I) and Petri dish (II). A: gravity catch; B: microscope slide; C: orifice (behind the slide or dish); D: slide support pin; E: Petri dish; F: carrier bracket.

## **Appendix B. Protocol to artificially wilt green citrus leaves to improve detection of *Guignardia citricarpa* in latently infected leaves**

1. Picked mature green leaves randomly from all four sides of the tree, with about 20 leaves per tree and from at least 20 trees per orchard block.
2. Keep detached leaves cool and process within six hours.
3. Wash leaves in running tap water to remove dirt and drain to remove excess water.
4. Air dry leaves for 12 hours out of direct sunlight OR air dry leaves for two to four hours in direct sunlight.
5. Soak air-dried leaves in tap water for 30 minutes, drain to remove excess water and place in a 20 µm thick clear plastic bag. Use 20 to 50 leaves per bag, depending on size of leaves and bag.
6. Closed bag, including as much as possible air within the bag, and place bag with leaves in an incubator at 42 °C for 6 h.
7. After 6 h, remove the bag from the incubator and mixed leaves by shaking the bag.
8. Open the bag to allow leaves to air dry and incubate under florescent and near-UV light for 18 h.
9. Repeat steps 5 to 8 for at least 21 days or until ample fructification of *Guignardia* is visible on the leaf surface.

Note: It is important to monitor the moisture within the bag closely, since no fungal fruiting structures will develop if the leaves are to dry and the leaves will rot if it is too wet. Unfortunately the correct moisture levels are only known through experience. Leaves have to air-dry completely on a daily basis to limit the growth of other fungi such as *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.