

TITLE: TECHNIQUE OF INDUCING OF SCLEROTIA FORMATION IN VITRO OF *Botrytis Squamosa*

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ABSTRACT:

Botrytis squamosa (Walker) is the etiological agent of Botrytis leaf blight of onion, characterized as the main leaf disease in the seedling stage of the crop. One of the primary sources of inoculum of this pathogen are the sclerotia. Works related to the survival and epidemiology of the primary inoculum of the disease require the purified organism and with expressive formation of sclerotia. The technique was developed at Instituto Federal Catarinense-IFC/Rio do Sul Campus with *B. squamosa* isolate obtained from conidia collected on injured leaf tissue of onion seedlings and multiplied for seven days at 25°C and without light in Petri dish containing PDA (Potato-Dextrose-Agar) culture medium. After this period, the conidia were removed from the mycelial growth by washing with sterile water. Using a hand spray, 2 mL of the 1x10⁵ conidia/mL suspension was sprayed into glass Petri dishes (90x15 mm) containing 20 mL of distilled water and 20 grams around of 1 cm long fragments of dry onion leaf straw previously autoclaved at 121 °C for 25 minutes. After inoculation, the Petri dishes were incubated in germination chambers of type B.O.D (Biological Oxygen Demand) for two weeks at 20°C without light. After this time, the fragments were withdrawn from the Petri dish and placed in a 300 ml glass stopper lid containing half a volume of water. After successive manual agitations the fragments were torn apart and the sclerotia detached from the fragments. Then, sclerotia and fragments were placed in a plastic sieve and with running water rubbed with the finger to remove fragments that could still be attached to the sclerotia. The sclerotia were removed from the sieve with the aid of forceps and placed in a Petri dish with an open lid for natural drying and subsequent storage at room temperature (25°C ± 2). The technique produces on average 200 sclerotia per Petri dish and their formation starts from the first week and stabilizes at 15 days of incubation. The morphological characteristics of the sclerotia produced by this technique resemble those produced by the plant in the field condition. This methodology has been used for the production of sclerotia used in epidemiological studies with the pathogen at the IFC/Rio do Sul Campus. This technique can be evaluated for sclerotia induction of other *Botrytis* species.

Keywords: Onion, technique, disease

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