ISOLATION AND CULTURING OF THE SMUT FUNGUS, *CONIODICTYUM CHEVALIERI*

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The smut pathogen, *Coniodictyum chevalieri,* was first described by Hariot and Patouillard in 1909 in Chad. It infects *Ziziphus mucronata* species, a multipurpose tree that occurs in Sub-Saharan Africa. The fungus was regarded as rare but in recent years, it was reported in South Africa, Senegal and Zimbabwe. In South Africa, an epidemic was reported in the Kruger Nation Park in 2004. The symptoms appear as white galls that are filled with teliospores and these appear on stems, branches, fruits and leaves (Fig.1).

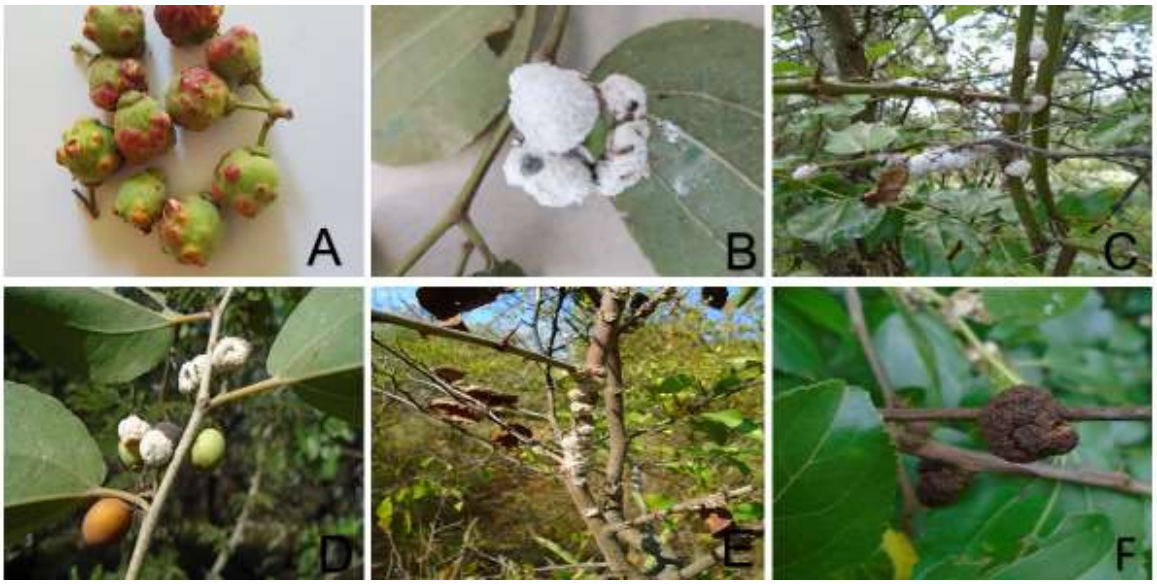

Fig. 1. Typical symptoms of smut disease caused by *C. chevalieri* on *Z. mucronata* species showing; (A) galls before they burst open, (B) open galls with white powdery teliospores, (C) galls on twigs, (D) galls on fruits, (E) wilting twigs due to disease and (F) old galls that have darkened

In order to carry out phylogenetic studies, isolation of the fungus was carried out from diseased samples collected from three sites in the Limpopo Province (Buzzard Mountain Farm, Tshikundamalema and Wits Rural Facility) as well as from Zimbabwe and Swaziland.

Smuts are semi-obligate parasites and therefore are difficult to culture on artificial media. They generally grow very slowly and the growth is usually hampered by the luxuriant growth of contaminants and other fast growing fungi. Spores were dusted onto the surface of malt yeast peptone agar in petri dishes. The petri dishes were incubated at 25 ^{o}C for 48h, after which single spores were transferred to malt yeast peptone agar and incubated at 25 ^{o}C. Single spores were mounted in lactic acid and examined using a Zeiss Axiosion microscope (Fig. 2).

Fig. 2. Spores of *C. chevalieri* viewed under a light microscope, showing irregular shapes and longitudinal panels

The colonies originating from single spores were transferred onto potato dextrose agar and again incubated at 25 ^{o}C. The colonies grew very slowly and after 5 weeks had reached 22.5mm in diameter with irregular shapes, lobate edges and raised elevation (Fig. 3). Some variation in the colour of the colonies was observed, depending on the age of the colony. The colonies started as light peach in colour with brainy appearance and after 5 weeks the edges turned white. Two distinct types of colonies were noted (Fig. 4). When viewed under a microscope, the spores also had irregular shapes but with distinct longitudinal panels.

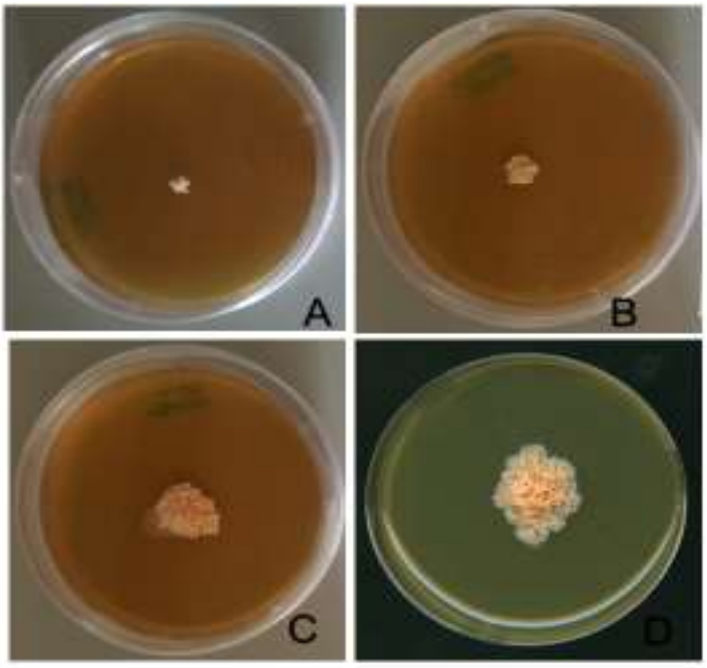

Fig.3. Growth of *C. chevalieri* colonies on potato dextrose agar over a 5-week period

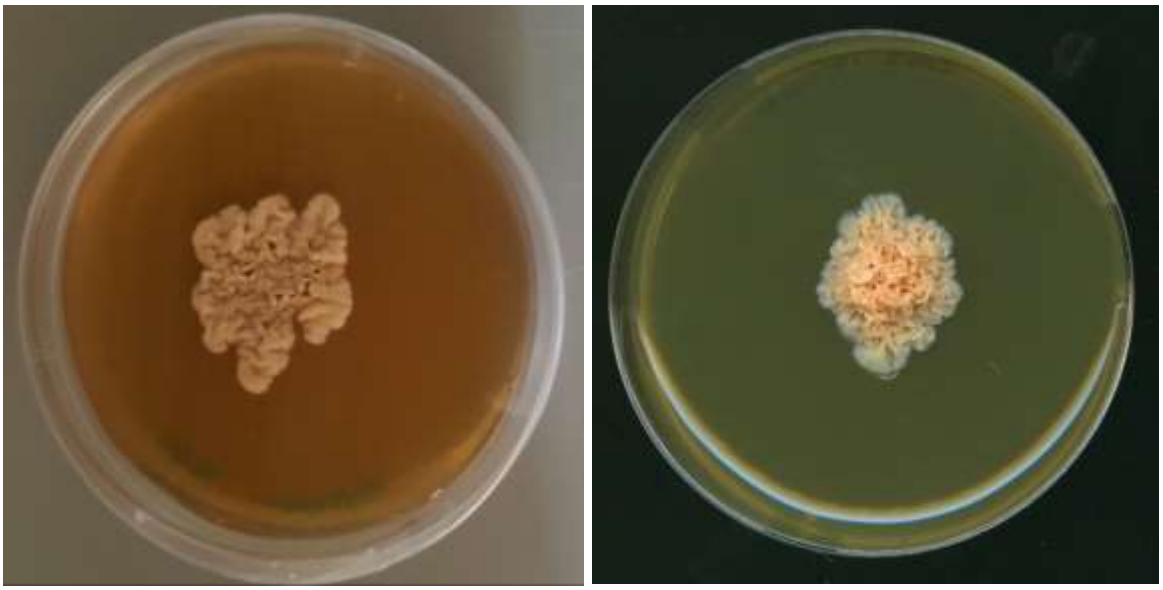

Fig. 4. *Coniodictyum chevalieri* colonies on potato dextrose agar, showing irregular shape and lobate margins