

# Koch's Postulates for New Species of Sooty Blotch and Flyspeck

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## Introduction

Sooty blotch and flyspeck fungi (SBFS) infect the cuticles of the apples resulting in dark blemishes on the surface. The number of species identified in the SBFS has increased with the help of molecular techniques. The following report summarizes the progress of confirming the identity of additional SBFS species using a modified Koch's postulates. Molecular techniques of DNA extraction and amplification were applied to fungi isolated from signs on apples.

## Materials and Methods

In 2005, a survey of apples in 30 orchards in the eastern United States revealed many new putative species of SBFS pathogens. These were morphologically characterized after isolation from the apples. The ITS region of rDNA of the extracted DNA was sequenced and for those that could not be amplified, a restriction digestion pattern was obtained.

In the summers of 2006 and 2007, mycelium suspensions from 1-month-old cultures of 100 isolates were used to inoculate young apples. This was done at the ISU Horticulture Station, Ames, IA on apple trees that were not subjected to fungicide sprays. Ten apples (1–3 cm) for each fungal isolate were arbitrarily selected on apple trees at mid-canopy level, surface sterilized with 70% ethanol, and swabbed with inoculum of a species using cotton swabs. The apples were covered by Fuji™ bag. After maturation (in mid-August to late September), the apples were harvested and using a dissecting

microscope, signs of the fungus on the surface were observed and recorded. Colonies from the apple were isolated and morphology was compared with the original isolate. Portions of the rDNA were also sequenced using the ITS1-F/ITS4 primers and compared with the original using the BLAST alignment tool. For isolates that did not amplify, restriction fragment length polymorphisms (RFLP) were obtained using the enzyme *Hae*III and compared with RFLP patterns from the original isolates.

## Results and Discussion

Of the 43 isolates inoculated in 2006, 13 were confirmed to be SBFS species. Of these, nine were confirmed through comparative alignment and four through RFLP. Of the 57 isolates inoculated in 2007, 25 were confirmed to be SBFS species, and work is in progress to confirm the others. All of the 25 confirmed were done using BLAST2, the alignment tool.

Low rates of success may be related to difficulty in reisolating fungi from signs on the apple and the fact that temperatures during inoculation were often above 30°C, which can be lethal to SBFS fungi.

Understanding more about the complex and its members will allow us to better manage SBFS and thus reduce the economic loss to the farmers.

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