

## Diseases Caused by Fungi and Fungus-Like Organisms

**First Report of *Trichoderma aggressivum* f. *aggressivum* Green Mold on *Agaricus bisporus* in Europe.** L. Hatvani, L. Kredics, H. Allaga, L. Manczinger, and C. Vágvölgyi, Department of Microbiology, Faculty of Science and Informatics, University of Szeged, H-6726 Szeged, Hungary; and K. Kuti, A. Geösel, and S. István, University, Faculty of Horticultural Science, Department of Vegetable and Mushroom Growing, H-1118 Budapest, Hungary. L. Hatvani and L. Kredics are joint first authors. This work was supported by grants NKFI K-116475 and GINOP-2.2.1-15-2016-00006. *Plant Dis.* 101:1, 2017; published online as <http://dx.doi.org/10.1094/PDIS-12-16-1783-PDN>. Accepted for publication 28 February 2017.

The white button mushroom (*Agaricus bisporus*) is the economically most important cultivated edible mushroom in Europe. The production of *A. bisporus* can be seriously affected by so-called green mold disease, caused by *Trichoderma aggressivum*. This species has two biotypes: *T. aggressivum* f. *europaeum*, being responsible for *Agaricus* green mold problems in Europe, and *T. aggressivum* f. *aggressivum*, the causal agent of the disease in North America (Samuels et al. 2002). *T. aggressivum* f. *europaeum* has been previously reported from Hungarian mushroom producing facilities as well (Hatvani et al. 2007). In December 2015, massive green mold epidemics occurred at a mushroom farm in central Hungary, cultivating the *A. bisporus* off-white hybrid with nearly 100% crop loss in the affected mushroom beds. The farm used Phase III bulk compost and the symptoms were visible before the first flush. Both the compost and casing soil were completely colonized by *Trichoderma*, with conspicuously visible mass of green conidia on the surface. In the same year, a further outbreak was documented at a mushroom growing company in northeast Hungary in the cultivation of *A. bisporus*. Compost and casing soil samples were collected at both locations and *Trichoderma* strains were isolated from the samples on dichloran-rose bengal agar medium. Colony morphology of the pure cultured isolates on potato dextrose agar and conidiophore morphology examined by light microscopy were consistent with the genus *Trichoderma* and the species *T. aggressivum* (Samuels et al. 2002). Molecular identification was performed at the species

and biotype level by sequence analysis of the ribosomal RNA internal transcribed spacer (ITS) region amplified by the universal primers ITS1 and ITS4 according to Andersson et al. (2009). Nucleotide BLAST analysis revealed that all isolates share the same ITS sequence showing 100% identity with the type strain of *T. aggressivum* f. *aggressivum* (DAOM 222156, GenBank NR\_137299.1), and differing in three diagnostic nucleotide positions from the type strain of *T. aggressivum* f. *europaeum* (CBS 100526, GenBank NR\_145035.1), as well as from previous Hungarian isolates of the European green mold biotype (Hatvani et al. 2007). To confirm the pathogenicity of the isolate to *A. bisporus*, six mushroom bags filled with 2 kg Phase III compost were inoculated with conidial suspensions ( $10^6$  conidia/ml; 50 ml/bag) of the Hungarian *T. aggressivum* f. *aggressivum* isolate SZMC 23834. The conidial suspensions were thoroughly mixed into the compost before casing and sprayed onto the casing soil at primordial stage. Commercial cultivation protocol was followed, while six uninoculated bags were used as controls. Twelve days after inoculation with SZMC 23834, the compost was almost black without visible *Agaricus* mycelia, while green spots were detected both in the compost and casing 14 days after inoculation. Fruiting body development into mature mushrooms was inhibited in the artificially inoculated bags. The underdeveloped fruiting bodies became covered by deep brown spots, while those developing on the uninoculated bags remained healthy. The compost infection by *T. aggressivum* f. *aggressivum* resulted in 100% crop loss, while 50% loss in the number of the fruiting bodies was recorded when the casing soil was treated. The pathogen was reisolated from compost and casing soil samples inoculated with *T. aggressivum* f. *aggressivum*. Ten recultured isolates were confirmed as *T. aggressivum* f. *aggressivum* based on ITS sequence analysis. To our knowledge, this is the first report of epidemic green mold outbreak in Europe due to the North American green mold biotype *T. aggressivum* f. *aggressivum*.

## References:

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