





GENETIC DIVERSITY AND PATHOGENICITY OF THE GREY MOULD FUNGUS *Botrytis*

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Resumen de la conferencia.

Genetic diversity and pathogenicity of the grey mould fungus *Botrytis*

Botrytis spp., causes pre- and postharvest decay on strawberry crops. *Botrytis* spp. isolates from several German strawberry-growing fields, which received several fungicide treatments against *Botrytis* per season, were analyzed to determine their sensitivity against botryticides. Fungicide resistance was commonly observed in *B. cinerea*, with many isolates possessing resistance to multiple fungicides. A stronger variant of the previously described multidrug resistance (MDR) phenotype MDR1, called MDR1h, was found to be widely distributed, conferring increased partial resistance to two important botryticides, cyprodinil and fludioxonil. A 3-bp deletion mutation in a transcription factor-

encoding gene, *mrr1*, was found to be correlated with MDR1h. All MDR1h isolates and the majority of isolates with resistance to multiple fungicides were found to be genetically distinct. Multiple-gene sequencing confirmed that they belong to a novel clade, called *Botrytis* group S. Isolates of *Botrytis* group S genotypes were found to be widespread in all German strawberry-growing regions but almost absent from vineyards. On the other hand, it was investigated the presence and fungicide sensitivity of *B. pseudocinerea*, which has been found previously to occur together with *B. cinerea* in low abundance in vineyards and strawberry fields. Abundance of *B. pseudocinerea* was often negatively correlated with fungicide treatments. On cultivated strawberries, it was frequently found in spring but was largely displaced by *B. cinerea* following fungicide applications. *B. pseudocinerea* almost never developed resistance to any fungicide even though resistance mutations occurred at similar frequencies in both species under laboratory conditions. Moreover, during the surveys carried out in the strawberry fields in Germany, some *Botrytis* isolates differed from *B. cinerea* and *B. pseudocinerea* in diagnostic PCR markers and growth appearance. Phylogenetic analyses showed these strains to belong to an undescribed species in *Botrytis* clade 2, named *Botrytis fragariae* sp. nov. Isolates of *B. fragariae* were detected in strawberry fields throughout Germany, sometimes at similar frequencies as *B. cinerea*. Various fungicide resistance patterns were observed in *B. fragariae* populations. Many *B. fragariae* isolates showed also resistance to one or several chemical classes of fungicides, and an efflux-based multidrug resistance (MDR1) phenotype previously described for *B. cinerea*. Resistance-related mutations in *B. fragariae* were identical or similar to those of *B. cinerea* for carbendazim (E198A mutation in *tubA*), azoxystrobin (G143A in *cytB*), iprodione (G367A+V368F in *bos1*) and MDR1 (gain-of-function mutations in the transcription factor *mrr1* gene, and overexpression of the drug efflux transporter gene *atrB*). The widespread occurrence of *B. fragariae* indicates that this species is adapted to fungicide-treated strawberry fields and may be of local importance as a gray mold pathogen alongside *B. cinerea*. Therefore, the sensitivity of the *Botrytis* population to botryticides should be frequently monitored in order to report shifts in sensitivities. The rotation and mixture with other site-specific or multi-site fungicides, such as thiram and captan, should be included to improve disease management in *B. cinerea* and *B. fragariae* populations.