Differentiation between *Botryosphaeria dothidea* and *Neofusicoccum* spp. through Detection of Single Nucleotide Polymorphisms in the ITS Region

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Abstract**—**The Botryosphaeriaceae family contains a large number of fungal species causing blight, cankers and fruit rot on a plethora of plant hosts. Among the most important hosts for the members of this family is pistachio (*Pistacia vera* L.). For the countries surrounding the Mediterranean basin, the main species responsible for high value losses in the pistachio cultivation are *Botryosphaeria dothidea, Neofusicoccum vitifusiforme, N. mediterraneum* and *N. parvum*. Since, too many species are implicated as causal agents in symptom development, the differentiation between them is of crucial importance. To resolve that, specific primers were designed on the ITS region utilizing detection of single nucleotide polymorphisms (SNPs) due to limited sequence variation within these species. Newly designed primers were combined with generic upstream ITS primers. Initially, downstream SNP primers were designed with a single 3' SNP base, T for *B. dothidea* and A for *Neofusicoccum* spp. However, reproducible and consistent differentiation proved difficult with these primers. As such, a different approach was implemented. Based on the initial SNP primers, new downstream SNP primers were designed. They included a mismatch next to the 3' base and a different 5' tail for each primer. The mismatch was different for each primer. The new approach was successful in the differentiation of the selected species. Further research regarding the design of rapid, reliable and cost efficient molecular markers for the identification between species of the Botryosphaeriaceae is underway. More specifically, Taqman primers and probes are being designed and will be evaluated on the mating type MAT1-2-1 and MAT1-1-1 gene sequences of *B. dothidea, N. mediterraneum* and *N. parvum*.

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Keywords**—***Camarosporium* blight, mismatch, molecular diagnosis, SNP.

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