Detection and quantitation of mixed infected samples with *Agrotis* specific baculoviruses

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Four baculoviruses, namely *Agrotis segetum* nucleopolyhedrovirus A (AgseNPV-A), *A. segetum* (Agse) NPV-B, *A.ipsilon* (Agip) NPV and *A. segetum* granulovirus (AgseGV) from the genera *Alpha*- and *Betabaculovirus*, respectively, are known to infect larvae of the lepidopteran pests *A. segetum* and *A. ipsilon*. The potential as biocontrol agents against *Agrotis* species benefits of two abilities, namely to crossinfect both pests *A. segetum* and *A. ipsilon* and to coinfect single larvae in mixed portions. In order to obtain a detailed understanding of mixed infections, especially between *Agrotis spp*. NPV and GV, and the amount of virus progeny produced, reproducible quantitative methods are necessary. This has been already set up with a SybrGreen-based RT-qPCR assay with specific primers binding in the core gene *polyhedrin*, or *granulin* respectively. By this method, mixed infections of AgseNPV-B and AgseGV in portions of their corresponding median lethal concentrations have been examined in experiments with neonate larvae of *Agrotis segetum*.

These findings revealed first hints to viral interactions between AgseNPV-B and AgseGV, as given by their potential virus progeny per larva. Ongoing experiments will consider investigations of mixed infections in cell culture studies with varieties of *Agrotis* specific baculoviruses and will be used for viral quantitation and microscopic picturing of mixed infections.

The SybrGreen-based RT-qPCR assay, however, requires a single RT-qPCR detection for each single virus and is thusly time- and sample-consuming. This lacks the ability for being used in experiments with more than two viruses, and/or in experiments with cell cultures of *Agrotis ipsilon*. An alternative assay with highly-specific *TaqMan* probes improves the quantification by a simultaneous RT-qPCR of up to four viruses in one reaction.