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## ÁREA VEGETAL E INVERTEBRADOS

### MOLECULAR DETECTION OF HONEY BEE VIRUSES IN APIARIES OF SOUTHERN BRAZIL

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#### Resumo

Bees are very important insects for agriculture, fulfilling an important role in pollination and renewal of the ecosystem. However, in several countries significant losses of colonies and population decline of honeybees and native bees have been reported, which are influenced by biotic and abiotic factors, including the effects of multiple pathogen infection and/or pesticide exposure. The majority of the viruses that have already been isolated and characterized in bees are classified as positive-sense single-stranded RNA viruses within the order *Picornavirales*, comprising the families *Dicistroviridae* and *Flaviviridae*. Prominent viruses include acute bee paralysis virus (ABPV), deformed wing virus (DWV), black queen cell virus (BQCV), sacbrood bee virus (SBV) and israeli acute bee paralysis (IAPV). Thus, the objective of this study was to detect the main bee viruses in apiaries in southern Brazil. Samples of honeycomb (larva and pupa) and adult bees were collected in the apiaries and kept under refrigeration until transportation to the Laboratory. Immediately the larvae and pupae were homogenized to TRIzol Reagent and frozen at -70 °C. Adult bees collected and stored in closed bottles were directly frozen. Six pupae, larvae and adult bee abdomen were submitted to RNA extraction and cDNA synthesis, followed by two multiplex polymerase chain reaction (PCR) (1: ABPV, CBPV and SBV; 2: BQCV, DWV and IAPV). All cDNA samples were tested with the endogenous control (GAPDH) to verify the efficiency of the whole process. RNA extracted from the bees' *pool* was used as negative control and gBlock® Gene Fragments were used as positive control of honey bee viruses. To date, 75 samples were obtained, mainly from southern Rio Grande do Sul, two of which were positive for IAPV (3,5%), three for ABPV (5,4%) and twenty-five for BQCV (33,3%), totaling 40% (30/75) of positive samples. All detected viruses were obtained from adult bees, and the identity of these viruses was confirmed by nucleotide sequencing. No viruses were detected in samples of larvae and pupae. These results demonstrate that ABPV, BQCV and IAPV viruses are present in apiaries in the South region of Brazil, with a high percentage of positivity for BQCV, and may, together with other factors, contribute for the bee population decline. This study is underway in order to increase the number of samples collected and phylogenetic characterization of the viruses detected.

## INTERCEPTION OF BARLEY STRIPE MOSAIC VIRUS-BSMV: A QUARANTINE VIRUS ABSENT IN BRAZIL DETECTED IN IMPORTED BARLEY GERMPASM

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### Resumo

*Barley stripe mosaic virus* (BSMV), a quarantine virus absent in Brazil, was intercepted in 2019 in imported barley (*Hordeum vulgare*) seeds. Expressive and typical symptoms of virus infection were observed in young leaves in seedlings from the abovementioned barley material (confidential process). The presence of BSMV in these symptomatic leaflets was confirmed by Enzyme-Linked Immunosorbent Assay (ELISA), using antibodies against BSMV (Agdia SRA 19500/0096) in biological and technical independent duplicates, resulting in clear detection of BSMV, once the spectrophotometry value was 1,063 times higher in barley samples than the experimental control samples. Visual inspection of symptoms and Polymerase Chain Reaction (PCR) for phytoplasma detection were negative. As BSMV is a regulated quarantine pest absent in Brazil (according to the Normative Instruction n. 39, 01/10/2018- Brazilian Ministry of Agriculture, Livestock and Food Supply), destruction of the respective imported seeds was recommended (incineration) and a report with technical information justifying such recommendation was issued. Samples of BSMV infected barley leaves were stored and will be used as positive control for future detection procedures. The presently reported BSMV quarantine interception of barley to be imported into Brazil reinforces the need for research on quarantine intelligence, development and validation of more sensitive molecular diagnostic methods, and preventive genetic improvement to combat the vulnerability of introduction of quarantine pests absent in the country, which can seriously jeopardize the primary sector of the national economy. This work is aligned with the Brazilian policy, which promote prevention and surveillance of quarantine absent pests, according to the "Portaria nº 131, 27/jun/2019 - Programa Nacional de Prevenção e Vigilância de Pragas Quarentenárias Ausentes (PNPV-PQA)".

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