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Insecticidal activity of two botanical extracts on *Aphis gossypii* Glover

Aphis gossypii Glover is one of the most important pests of crop and vegetable plants in Asia and Europe. However, such sufficient knowledge of insecticidal activity of botanical insecticides on aphids is still lacking in the literature. Therefore, the efficacy of acetonic leaf extracts from *Otostegia persica* (Labiatae) and *Calotropis procera* (Asclepiadaceae) were evaluated using 3 to 4 days-old individuals of the *A. gossypii*.

In order to obtain the crude extracts, the dried leaves were extracted with aceton. Water and DMSO (Dimethyl sulfoxide) were used as control treatments. Topical treated aphids with two acetonic extract emulsion (in distilled water with DMSO) were placed on the broad bean leaf discs (4.5 cm diameter) in the round plastic Petri dishes (5.5 cm diameter), filled with a 0.5 cm-thick agar gel layer. The highest percentage of mortality (66 %) was observed in the acetonic leaf extract of *O. persica* in the concentration of 70 µL/l. While, the percentage of mortality (58 %) was recorded in the *Calotropis* leaf extract in 100 µìL/l. It could be concluded that these plant extracts may be applicable as a safe insecticide to control *A. gossypii*.

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Comparison of methods for monitoring the resistance of codling moth populations to *Cydia pomonella* granulovirus (CpGV)

In organic as well as integrated apple production the *Cydia pomonella* granulovirus (CpGV) is the most successful biological control agent to control the worldwide occurring pest *Cydia pomonella* (codling moth, CM). It is an efficient tool to reduce fruit damages and CM population densities of the following generations and is therefore often applied in combination with chemical insecticides. All commercial products contain the same isolate CpGV-M. CpGV is applied in Germany for nearly 20 years, but since first local observations of CM populations resistant to CpGV-M had been reported (Nachrichtenbl. Dt. Pflanzenschutzd. 57, 29-34 (2005)) various approaches were made to elucidate the resistance mechanism (Science 317, 1916-18 (2007)) and to select new different CpGV isolates, e.g. CpGV-I12, which overcome the CpGV resistance of CM (J. Invertebr. Pathol. 98, 293-98 (2008); Appl. Environm. Microbiol. 75, 925-30 (2009)).

However, implementation of resistance monitoring including fast prediction of potentially resistant field populations is a prerequisite for an effective resistance management. In this study two different methods for monitoring CpGV resistance were evaluated:

In method A, neonate offspring (F1 generation) of diapausing larvae, collected from local field populations in late summer, were submitted to bioassays to estimate their susceptibilities to CpGV-M. In addition, coherent units of the F1 generations were maintained in a laboratory rearing on artificial diet for further investigations. Bioassay diet was mixed with virus at concentration ranging from 10^3-10^8 OB/ml diet and the plates were stored at 26 °C and 16/8 h photoperiod. Larval mortalities were determined after 7 and 14 days and the lethal concentrations (LC_{50}) were calculated from concentration mortality curves using a probit analysis. From 2003 to 2008 a systematic survey of several organic orchards in Germany indicated the existence of different resistance levels (LC_{50} values of 10^5-10^8). In 2008, two populations from different orchards although treated with new resistance overcoming virus isolates, proved to be resistant to these viruses. The used method allows an accurate determination of different resistance levels of local CM populations. LC_{50} values and their corresponding slopes of the probit regression lines reveal the heterogeneity of a population. A disadvantage of this method is the hibernation of insects (up to nine months), which is rather time consuming and labour- and cost-intensive. Furthermore the results of the tests are available only in the following year and thereby the prognosis of evidence of resistance is delayed.

Therefore, a rapid test system (method B) was developed using first to fourth instar larvae removed directly from infested apples for bioassay treatments with a single discriminating concentration (2 x 10^5 OB/ml diet) of CpGV-M or CpGV-I12. This virus concentration caused 95-98 % mortality of CpGV-M sensitive larvae and approximately < 30 % mortality of resistant individuals in 14 days bioassays (conducted as described for method A). This method provides a very fast screening (in average 3 weeks) of resistant field populations. From 2007 to 2009 about 14000 apples from 32 different orchards in Germany, Austria, Switzerland, Italy and The Netherlands were examined for larvae and their susceptibility to CpGV-M.

During the studies seven populations were identified as resistant to CpGV-M. Further 12 populations showed 100 % mortality to the resistance overcoming CpGV-I12. The described method is appropriate to give a reliable and fast

prognosis of CpGV resistant populations in the field. However, disadvantages are that only few insects can be tested without any repetition of trials. Thus, both methods have pros and cons: for detailed analysis method A is superior, if fastness and high-throughput is needed, then method B is more efficient.

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The genomic variety of CpGV isolates: comparison of four genotypes

The *Cydia pomonella* Granulovirus (CpGV) is an economically important agent for the biological control of codling moth (*Cydia pomonella*, CM). Recently, the emergence of CM populations highly resistant to CpGV products (Science 317, 1916-18 (2007)) as well as the identification of new CpGV isolates overcoming CpGV resistance (J. Invertebr. Pathol. 98, 293-98 (2008)) were reported. Here we describe the genome sequencing and comparative genomic analyses of CpGV isolates vulnerable to resistance (CpGV-M) and of isolates overcoming resistance (CpGV-I12, -S) as well as an isolate with reduced virulence to susceptible CM larvae (CpGV-I07).

The isolate CpGV-M1 was one of the first fully sequenced granulovirus genomes. By restriction fragment length polymorphism (RFLP) analysis, further CpGV isolates had been previously identified and were designated due to their geographic origin. Applying phylogenetic analysis of ten CpGV isolates based on the polyhedrin/granulin (polh/gran) and late expression factor-8 (lef-8) genes, CpGV isolates could be recently grouped into genome types A to E, replacing the previous classification. To gain insight into the genomic variety and plasticity of CpGV genomes, four CpGV genome types were completely sequenced: CpGV-I12 (type D genome), -S (type E genome), -I07 (type C genome) and compared to CpGV-M (type A genome), which was re-sequenced as reference. Genome analysis revealed differences in genome size and genetic content between the four isolates. Several insertions and deletions ranging from few nucleotides to 2.5 kbp were found, concerning non-coding as well as putative coding regions. Regarding the site of these indel mutations, it is striking that the genome regions between 18-22 kbp and 50-60 kbp reveal a multiplicity of insertions, deletions and duplication events when comparing the four genomes, suggesting that these events are associated with the homologous repeat (hr) regions. Analysis of these genomic rearrangements, open reading frame (ORF) content and codon usage give insight into the evolutionary forces driving the micro-evolution of baculovirus genomes. As type D and type E genome overcome the previously described resistance of codling moth to CpGV), the comparisons of the four genomes revealed first evidence for the molecular factors involved in the virulence of CpGV to susceptible and resistant codling moth.

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Development of a PCR based method for identification, discrimination and quantification of baculoviruses specific for cutworms, *Agrotis* sp.

Cutworms of the species *Agrotis segetum* and *A. ipsilon* (Lepidoptera, Noctuidae) are serious pest insects in Africa, Europe and Asia, as they feed on many field crops and vegetables.

In the past, four baculoviruses were isolated from *A. segetum* and *A. ipsilon* larvae and characterized on molecular level: Two nucleopolyhedroviruses (NPVs) were isolated from *A. segetum* larvae in Poland (AgseNPV-A) (J. Invertebr. Pathol. 90, 64-8 (2005)) and United Kingdom (AgseNPV-B) (Arch. Virol. 75, 43-54 (1983)), one AgipNPV (J. Invertebr. Pathol. 74, 289-294 (1999)) was found in *A. ipsilon* larvae and a granulovirus (AgseGV) was also isolated from *A. segetum*. Bioassays showed that both cutworm species are susceptible to all AgseNPV-A, -B, AgipNPV and AgseGV. To develop an environmentally safe biocontrol agent the narrow host range of baculoviruses is one of their advantages. For resistance management, however, the usage of a combination of different baculoviruses is regarded to be useful. Both requirements make AgseNPV-A, -B, AgseGV and AgipNPV excellent candidates as agents for the biological control of cutworms. In order to discriminate the different *Agrotis*-specific baculoviruses a reliable method for identification and quantification is essential.

In this work, we focused on the optimization of AgseNPV and AgseGV purification protocols and show that the yield of NPVs and GVs in mixed infections depends on the established purification method. Furthermore, multiplex polymerase chain reaction (PCR) and quantitative real time PCR (qRT-PCR) based methods were established allowing the specific amplification of discriminating fragments of their polyhedrin (polh) and granulin (gran) genes (fragment lengths: AgseNPV-A 199 bp, AgseNPV-B 263 bp, AgseGV 347 bp and AgipNPV 527 bp). Thus, a rapid and robust method to detect the amounts of AgseNPV-A, -B, AgseGV and AgipNPV in mixed infections becomes possible. It also provides an important tool in the quality control of production of baculoviruses specific for *Agrotis* species.