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Marc Sporleder "The granulovirus of the potato tuber moth *Phthorimaea opeculella* (Zeller) – characterization and prospects for effective mass production and pest control", University of Hohenheim, 2003

Summary

The potato tuber moth, *Phthorimaea operculella* Zeiler (Lepidoptera: Gelechiidae), is a cosmopolitan pest causing significant damage on potatoes *(Solanum tuberosum* L.) and other solanaceous crops in tropical and subtropical regions. Faced with serious and increasing problems due to the extensive use of chemical pesticides to control this pest, safer alternatives such as the use of entomopathogens are under investigation to replace toxic chemicals in integrated pest management (IPM) strategies. Among them the highly host-specific granulovirus infecting *P. operculella* (PoGV) has shown prospective potential to reduce infestations during storage and in potato fields.

The objective of the present study, which was carried out in the International Potato Center (CIP) in Lima, Peru, within the project "Developing IPM for the potato tuber moth in cropping systems of different agroecological zones" funded by the Federal Ministry of Cooperation and Development (BMZ), was to enhance the feasibility using PoGV as a biopesticide through improving mass propagation techniques and development of appropriate formulations for improving UV-stability. Additionally, the granulovirus-host insect interaction and how the system is influenced by environmental factors were studied to gain knowledge on the appropriate utilization of *PoGV* in different agroecological zones. This included the biological and physical characterization of different geographical PoGV isolates and life cycle studies on the insect host itself.

A procedure to assess infectivity (LCso) is crucial in the research of pathogens. The egg-dip bioassay technique developed in this study by using purified *PoGV* proved high precision, is easy to reproduce and can be recommended as a standard bioassay. Temperature did not directly affect the infectivity of the virus (LC_{50}) but the development time and mortality of the host. Hence, the virus should be propagated in zones where temperature exceeds at least 20°C, better 24°C, to obtain rapid development and minimize natural mortality rates of the host *P. operculella*. The relationship between mortality response (LC_{50}) of *P. operculella* to *PoGV* and larval age (larval weight) was also determined. Susceptibility of *P. operculella* to *PoGV* decreases with larval age, with a linear relationship between log LC_{50} and log larval weight. The larval weight could be related to the physiological age calculated as degree-days. The established model has value to predict the efficiency of PoGV applications in the field. The speed of change in susceptibility of P. *operculella* to PoGV through selection of survivors from virus-infected larvae over 12 generations was quantified in three moth populations using sub-lethal PoGV concentrations of 10^8, $2.5x10^7$, $6.25x10^6$ granules/ml. Susceptibility was reduced to 55.3%, 56.4%, and 62.6% in each subsequent infected generation, respectively. After 12 generations all three populations were virtually resistant against the virus. The resistance remained after one generation of backcrosses with the susceptible parental population. This does not necessarily mean that resistance to PoGV will be a problem in the

field, since rapid revision of resistance has been noted in various other insect-baculovirus systems. With extended use of PoGV research should also be directed to determine the possibilities **of** development of resistance by field populations of *P. operculella* to viral insecticides to enable implementation before resistance becomes a problem.

The biological activity of 14 *PoGV* geographical isolates was assessed against a Peruvian host population using the egg-dip bioassay technique. The differences in biological activity (LCsol were significant, ranging from $2.86x10^6$ granules/ml ("La Molina") to $9.5x10^9$ granules/ml ("Yemen"). Genomic polymorphisms were detected in the isolates "Kenya" and "Huaraz" using restriction enzymes *HindIII* and EcoRI, respectively. Propagation of the isolate "La Molina" can be recommended within Peru, while in other countries the most potent available isolate should be identified by testing against local host populations before recommendations can be made.

The development, immature mortality, and reproduction of *P. operculella* were studied at different constant temperatures within the range of 10-32°C. The data were used to build £ P. *operculella* population model (rate-summation model) based on temperature, and life table parameters simulated over a range of temperatures gave good predictions when compared with literature data. The model is not only helpful for optimizing the *Po*GV mass propagation process and application time, but also for estimating the pest incidence in different agroecological zones.

In a series of four multi-factorial experiments the effects of infective inoculum, the larval rearing density, the incubation temperature and the type of food presentation (potatoes) were evaluated for maximization in the *PoGV in vivo* production. Egg inoculation with purified *Po*GV using 10^9 granules/ml was best. 2 g potatoes per larva were optimal for both production of host insects and for virus multiplication. Rearing temperature within the range of 25-28°C yielded highest rates of virus infected larvae as well as rapid larval development and can be recommended for PoGV mass propagation units. Virus propagation under cooler conditions (i.e. Andean highlands) can be improved by presenting potato slices instead of intact potatoes and by increasing the larval rearing density to compensate for the increased natural mortality of P. *operculella* (loss in larval yields) under cooler conditions.

With the option to obtain synergisms, the mode of interaction between *Po*GV and *Bacillus thuringiensis* var. *kurstaki* Berliner *(B.t.)* and between *Po*GV and azadirachtin was determined in bioassays. The interaction between *Po*GV and azadirachtin was mixed, but tended towards synergism, while it was antagonistic between *Po*GV and *B.t.*

A bioassay method to assess *Po*GV inactivation due to natural radiation was developed and tested at different intensities of natural solar irradiation in Lima (240 m a.s.l.), and in the Peruvian Andes at different altitudes to gain insight into the kinetics of inactivation and the effect of altitude due to changing (UV-) light spectrum. Dry deposits of *Po*GV were exposed and irradiance measured by using a pyranometer and a UV-B sensor. A bisegmented curve of inactivation with an initial steep decline followed by a much shallower part, both quite well described by two individual exponential curves with half-life times of several minutes, was found. In terms of total energy in Lima an average of $308x10^3$ joules/m^2 (by record of the pyranometer) caused 50% reduction of the viral activity. The curve curtailed when approxi-mately 98% of the virus had been inactivated. Thereafter inactivation was 4.4 times slower (l,$370x10^3$ joules/m^2). A modified two-component model described by HIATT (1964) could be successfully fitted to the total set of data. Speed of inactivation increased by 2.8%, while the proportion of UV-B in the sunlight increased only by 1.4% with each rise of 100 m in altitude.

To find out the protective capacity of UV adjuvants as well as their effects on the viral activity, several dyes, antioxidants and two types of formulations — a wettable powder (WP) and a soluble concentrate (SC), each type without and with UV screen - were examined in experiments with exposure of dried virus deposits to natural sunlight. Congo Red (dye) was detrimental to viral activity. The optical brightener Tinopal LPW enhanced the viral activity at concentrations above 0.01% by factors ranging from 8.6 to 134. Higher mortalities obtained from irradiated *Po*GV with Tinopal compared to unprotected *Po*GV might be attributed to enhancement of remaining viral activity rather than to increased virus persistence. The antioxidants propyl gallate and phenylthiocarbamide increased the viral activity by factors of 18 and 6, and increased 2- and 3-fold the viral stability, respectively. *Po*GV with host derived material (macerated virus-infected larvae) displayed 6-fold increased stability compared to purified *Po*GV. *Po*GV formulated as a WP showed persistence 1.4 to 2.5 times higher than unprotected *Po*GV. No significantly increased virus stability was observed due to the SC formulations.

The activity and solar stability of purified *Po*GV applied to potato foliage was determined by assaying leaf samples during two experiments under different natural irradiation. In the second experiment the protective capacity of a WP and a SC formulation - with and without sun-screens - was tested. The LC_{50} of purified *PoGV* on potato leaves ranged from 2.8x10 to $1.6x10^6$ granules/ml. The activity was reduced to 6.5 and 9.2% by one day of exposure (half-life times of 0.25 and 0.29 days) due to sunlight in the two experiments. Thereafter half-inactivation times reduced to 2.3 and 2.8 days in the first and second experiments, respectively. In the first experiment 1.4% of the activity remained after 6 days of exposure, while in the second experiment a further rapid inactivation (half-life: 0.33 days) from the 4^{th} to the 6^{th} day occurred and only 0.2% of the activity remained at the end of the experiment. The probi regression lines obtained from formulated virus showed reduced slopes compared to *PoG*\ alone. No capacity to reduce virus inactivation was observed in the SC formulation. The SC formulation, which included sunscreens, showed approximately 10-fold increase of virus activity. The activity of the virus in both WP-formulations remained high up to the 4^{th} day of exposure to sunlight (>50%), but remaining activities by the 6^{th} day of exposure were lower than in any of the other treatments (<0.001 %).

As a final conclusion it can be said that *Po*GV has prospects for successful implementation into IPM strategies as a selective biopesticide enabling replacement of toxic chemical components in these strategies. The obtained data allow differentiation between condition occurring in different agroecological regions for appropriate realization and decision-making.

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