Differences in the susceptibility of codling moth populations to *Cydia pomonella* granulovirus in the Czech Republic

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Abstract

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The *Cydia pomonella* granulovirus is a very important agent for the biological control of the codling moth, *Cydia pomonella*, in both organic and integrated apple and pear production. Three populations of *Cydia pomonella* originating from three separate areas of the Czech Republic were tested for their susceptibility to *Cydia pomonella* granulovirus in laboratory bioassays at several concentrations of *Cydia pomonella* granulovirus. A sensitive laboratory strain was chosen as a control. The larval mortality was checked 14 days after the infection. The mortality of *Cydia pomonella* larvae was similar in specimens originating from both the wild populations and the laboratory strain. Decreased susceptibility to *Cydia pomonella* granulovirus was demonstrated neither in samples from locality without *Cydia pomonella* granulovirus treatment nor from a locality experimentally treated with *Cydia pomonella* granulovirus for several years during the registration process. However, one population experimentally treated for more than 10 years was partially resistant to *Cydia pomonella* granulovirus. Based on our findings; the *Cydia pomonella* granulovirus biopesticides will be efficient due to the high susceptibility of field codling moth populations to *Cydia pomonella* granulovirus in the Czech Republic.

Keywords: codling moth; *Cydia pomonella* granulovirus; resistance; biological control; baculovirus

Generally, baculoviruses are the major group of arthropod viruses studied for their biological potential to control pests in agriculture and forestry (Szewczyk et al. 2006). Considering their high environmental stability, virulence, host specificity and the safety to human health, non-target organism and the lack of negative influence on the environment, baculoviruses are used as efficient bioinsecticides (Hunter-Fujita et al. 1998; Asser-Kaiser et al. 2007). The host range of each baculovirus is usually limited to a few closely related species, especially from the insect orders Lepidoptera, Hymenoptera, and Diptera (Moscardi 1999). *Cydia pomonella* granulovirus (CpGV) is highly pathogenic for the codling moth (*Cydia pomonella* L.) and its infection results in high mortality of early instar larvae. Infected larvae succumb within 5 to 10 days (Jehle et al. 2006; Eberle et al. 2008).

In the late 1980s, the first CpGV biopesticide was registered for commercial use in some European countries. Several CpGV biopesticides are now commercially available in most European countries, in North America, Argentina, New Zealand, and South Africa (Lacey, Shapiro Ilan 2007). In the Czech Republic, two CpGV based biopesticides called Madex (Andermatt Biocontrol AG, Switzerland) and Carpovirusine (Natural Plant Protection, France) were recently registered based on the results from semi-field experiments.

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While the registration process in the Czech Republic has just finished, Germany, France, Switzerland, Italy, and Netherlands already reported the occurrence of several populations of codling moth resistant to CpGV (Jehle et al. 2006; Jehle 2008). Studies on the mechanism of inheritance of CpGV resistance suggest that it was effectively selected in the field by applying single CpGV isolate (Asser-Kaiser 2007; Jehle 2008).

The Czech populations of codling moth have not been exposed to massive application of CpGV. However, CpGV-based preparations were applied experimentally in several locations in the Czech Republic for the registration process (e.g. Stará, Kocourek 2003). We expect the absence of resistant strains, but the situation could change after registration and massive applications of CpGV. Here, we report the natural susceptibility to CpGV of selected codling moth wild populations from the Czech Republic in laboratory bioassays.

MATERIAL AND METHODS

The origin of wild strains moths

The overwintering larvae of codling moth from field populations were collected using paper belt traps on three areas of the Czech Republic in the autumn of 2007 and 2008: (i) apple orchard in Velké Bílovice; (ii) apple alleys in Bulhary (only in 2007); and (iii) apple alley and apple orchard (only in 2008) in Prague-Ruzyně. The apple orchard in Velké Bílovice (48°51'N, 16°54'E) is located in South Moravia near Brno. It is part of an intensive apple growing farm under the integrated pest management (IPM) regime since 1992. In this orchard (VB I 08), the larvae were collected in the autumn of 2007 at a distance of 600 m from a plot experimentally treated since 1997 in accordance with the registration process of CpGV-based biopesticides. The larvae were collected also in the summer of 2008 from injured apples and in the autumn of 2008 from cardboard belt traps directly in the plot treated with CpGV based biopesticides (VB II 08, VB II 09). The Bulhary (B 08) alley (48°49'N, 16°44'E) is located in South Moravia along the road near to the Bulhary village and it has never been treated with chemical pesticides or CpGV. The Prague-Ruzyně (P I 08, P I 09) alley (50°06'N, 14°15'E) is located in Prague and belongs to the Crop Research Institute. This alley has never been treated with chemical pesticides or CpGV, but it is 400 m away from the experimental orchard (P II 09) treated in 1998–2000 and 2006–2008 with CpGV-based biopesticides in accordance with the registration process.

The larvae collected in the autumn of 2007 and 2008 were stored for 4 months in a climate chamber at 6°C. At the beginning of the next year, the temperature in the climate chamber was gradually increased to 20°C. Adults of codling moth hatched during the spring and they were put into a box situated in the glasshouse with natural light conditions for ovipositioning. Branches of apple trees were placed around the boxes to simulate natural conditions and promote the oviposition (Witzgall et al. 2005). The larvae collected from apples in the summer of 2008 were cut out of the apples, placed on an instant artificial diet (Manduca Premix-Heliothis Premix, Stonefly Industries, KS, USA) and transported from the orchard to the climatic chamber with 26°C. Some of them pupated, hatched and were transported to the oviposition box.

The laboratory susceptible strain

We used the susceptible Crimean laboratory strain of codling moth as the control of our experiments. This strain originated in Russia (Vsesajuznyj Institut Zascity Rastenij, Saint Petersburg) and has been maintained in the Crop Research Institute since 1992. The strain is reared on an artificial diet under standard conditions of 26°C, 60% relative humidity, and a photoperiod of 16:8 h (L:D).

Bioassay

We adopted the bioassay described in Fritsch et al. (2005, 2006); eggs laid on parafilm by females were collected daily and incubated at 25°C. Neonate larvae (L1) were transported using a paintbrush and placed individually on the surface of the instant artificial diet (Manduca Premix-Heliothis Premix, Stonefly Industries, KS, USA). A 30 ml water suspension of the CpGV-M was mixed with 10 g of Premix diet to obtain concentrations of CpGV-M from 300 to 300,000 occlusion bodies per ml (OB/ml) the diet (laboratory strain of codling moth) and from 1,000 to 300,000 OB/ml (wild populations of codling moth). A purified stock of CpGV-M originating from DLR Rheinpfalz (Germany) was used for the bioassay. The number of OBs/ml in

Table 1. CpGV-M concentrations tested against the sensitive laboratory strain and against the wild populations from Bulhary, Prague-Ruzyně, and Velké Bílovice and the number of CpGV-M concentrations in the bioassay

Strain/population	Term of larvae collection	Year of bioassay	Number of larvae tested	CpGV-M concentration range (OB/ml)	Number of CpGV-M concentrationin the bioassay
Laboratory strain	–	2008	443	3×10^2–3×10^5	7
Bulhary (B 08)	autumn 2007	2008	173	10^3–3×10^4	4
Prague-Ruzyně (P I 08)	autumn 2007	2008	44	10^3–3×10^4	4
Velké Bílovice (VB II 08)	summer 2008	2008	104	10^3–3×10^4	4
Prague-Ruzyně (P I 09)	autumn 2008	2009	100	3×10^5	1
Prague-Ruzyně (P II 09)	autumn 2008	2009	149	3×10^5	1
Velké Bílovice (VB II 09)	autumn 2008	2009	1,096	3×10^5	1

this stock was scored using a Petroff-Hauser counting chamber (depth 0.02 mm) in a dark field optic microscope (Olympus CX 41 RF, Olympus Optical Co., Ltd., Japan).

The larvae were reared in polypropylene (PP) autoclavable boxes with 50 places (File Cases for Micro Vials, Cat. No. 2-1535, NeoLab, Germany) covered with filter cap plugs and incubated in a climatic chamber at 26°C and a photoperiod of 16:8 h (L:D). The number of larvae tested and the CpGV-M concentrations in the bioassay are given in Table 1. The larvae that died after one day of infection were removed because their death was likely a consequence of injury during manipulation. The mortality of larvae in the bioassay was evaluated symptomatically 14 days post infection.

Statistical analysis

Mortalities of the larvae from the laboratory strain of codling moth were analyzed by the Probit analysis (XLSTAT 2008, Addinsoft, USA). The 50% (LC50), 90% (LC90), and 95% (LC95) lethal concentrations, 95% confidence intervals and slopes and intercepts of the dose-response curves were determined for the laboratory strain. Against the laboratory strain, seven CpGV-M concentrations were tested (3×10^2–3×10^5 OB/ml). Because of a low number of larvae of wild populations available for full range bioassay, only four CpGV-M concentrations (10^3–3×10^4 OB/ml) were used in 2008. In 2009, the CpGV-M concentration 3×10^5 OB/ml causing the 100% mortality of sensitive laboratory individuals 7 days after the start of the bioassay was used. Regarding the low number of CpGV-M concentrations tested, the susceptibility of wild populations to CpGV-M was evaluated by comparing the mortality of wild population in the probit with the confidence interval of the dose-response curve of the laboratory strain.

RESULTS AND DISCUSSION

Laboratory strain of codling moth

The average mortality of the laboratory strain larvae at one day post infection (mortality due to manipulation) was 0%. In the untreated control variant, the mortality of larvae 14 days post infection was 0%. The concentration-mortality response curve of the laboratory strain evaluated at 14 days post infection is presented in Fig. 1. It is evident that the mortality of larvae increased with the concentration of CpGV-M ($P < 0.001$, $\chi^2 = 247.3$, $df = 1{,}442$). At fourteen days post infection, the CpGV-M had a high efficacy against larvae from the laboratory strain with LC50 of 0.99×10^3 OB/ml, LC90 of 4.57×10^3 OB/ml and LC95 of 7.04×10^3 OB per ml of diet. This result was comparable with the recent finding of Fritsch et al. (2005, 2006) and, confirmed the susceptibility of the laboratory strain of codling moth to CpGV-M.

Field populations of codling moth

The average mortality of codling moth larvae derived from the Bulhary (B 08), Prague-Ruzyně (P I 08, P I 09, P II 09), and Velké Bílovice (VB I 08, VB II 08, VB II 09) wild populations at one day post infection

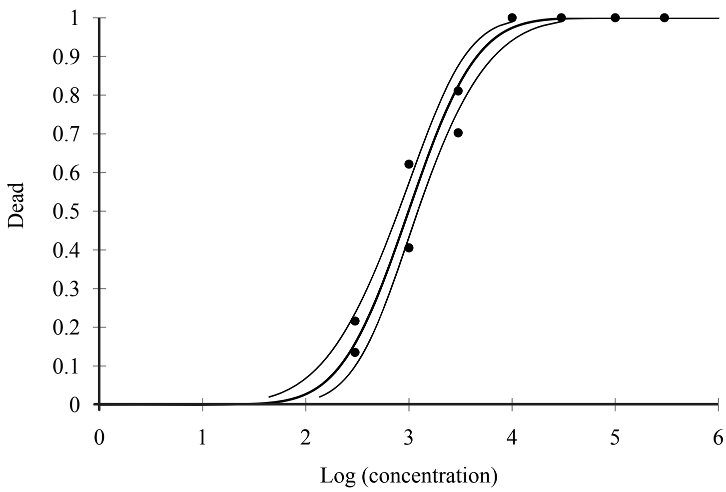

Fig. 1. Logistic regression of dead by Log (concetration). Concentration-mortality response curve of the laboratory strain (black line) evaluated at 14 days post infection. Each black dot represents the mortality recorded from 70 test larvae. Fine black lines indicate 95% confidence intervals

did not exceed 10%. The mortality in the control variant without CpGV-M at 14 days post infection was 0%. The mortality of larvae from wild populations (B 08, P I 08, VB II 08, VB II 09) in relation to the log of CpGV-M concentrations is presented in Fig. 2. However, according to the position of points corresponding to mortality of larvae from the tested wild populations in the Fig. 2 with respect to the confidence interval of the concentration-mortality response curves of the laboratory strain, the susceptibility of wild populations from Bulhary and Prague-Ruzyně did not differ significantly from the laboratory strain (in most cases, the mortality rate of wild populations is inside the confidence interval of the concentration-mortality response curve of the laboratory colony). The susceptibility of the codling moth population from Velké Bílovice to CpGV-M differed depending on the place of larvae collection. The larvae collected in 2007 in the plot without CpGV treatment (VB I 08) were susceptible. The mortality of 30 larvae from this plot reached 100% when exposed to CpGV-M in a concentration of 10^4 OB/ml (log *c* 4.00) and 3 × 10^4 OB/ml (log *c* 4.48) (data not shown). In contrast, 83.3% and 63.6% of the progeny of codling moth derived from larvae collected directly from apples in the summer 2008 (VB II 08) in a plot treated with CpGV-based biopesticides since 1997 survived the exposure to CpGV-M in a concentration of 10^4 OB/ml and 3 × 10^4 OB/ml, respectively. Moreover, on average

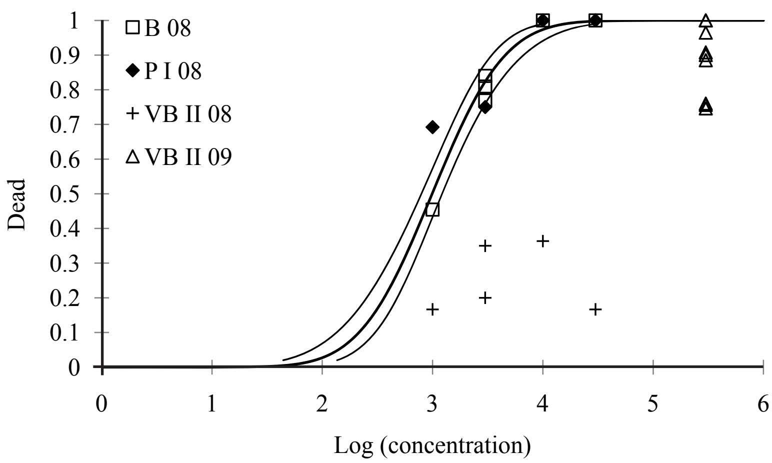

Fig. 2. Logistic regression of dead by Comparison of efficacy of CpGV-M tested against the sensitive laboratory strain and against the wild populations collected in the autumn of 2007 in the apple alleys of Bulhary (B 08) and Prague-Ruzyně (P I 08) and collected in the summer of 2008 and the autumn of 2008 in the orchards of Velké Bílovice (VB II 08*, VB II 09*) evaluated at 14 days post infection. Fine black lines indicate 95% confidence intervals, black line indicate concentration-mortality response curve of the laboratory strain (*collected from the CpGV treated part of orchard)

11.3% of the progeny of codling moth collected in this plot in the autumn of 2008 (VB II 09) survived the exposition of CpGV-M in a concentration of 3×10^5 OB/ml. According to Schmitt et al. (2008), codling moth larvae surviving the infection by CpGV-M in concentration of 10^4 OB/ml (evaluated at 14 days post infection) can be considered resistant. In our experiments, the larvae from Bulhary, Prague-Ruzyně, and Velké Bílovice originated from plots without the CpGV treatment had always 100% mortality 14 days post infection with a virus concentration of 10^4 OB/ml, 3×10^4, and 3×10^5 OB/ml. No decreased susceptibility to CpGV-M was recorded in the samples of codling moth populations originating either from the locality without CpGV treatment (B 08) or 400 m away (P I 08, P I 09) from CpGV treated plots. The same results, 100% mortalities of larvae 14 days post infection in 3×10^5 OB/ml were observed in larvae of Prague-Ruzyně orchard (P II 09). The susceptibility did not decrease either, despite the fact that this orchard was experimentally treated with CpGV. However, the resistance was observed in samples of codling moth population originating directly from the plot in the Velké Bílovice orchard experimentally treated for 12 years with CpGV-based biopesticides (VB II 08, VB II 09; Fig. 2). No decreased susceptibility to CpGV was found in codling moth samples from plots located 600 m (VB I 08) away from CpGV treated plots. Yet, the low number of tested individuals from this plot does not enable to conclude the status of susceptibility of this population to CpGV.

Several research institutes and producers currently search for the main reasons of the origin of the resistance as well as possible solutions of this situation (reviewed by Jehle 2008). Andermatt Biocontrol AG laboratories managed to obtain a virus strain efficient against the resistant codling moth populations by subsequently passaging CpGV-M through the resistant population. The commercial name of this new biopesticide is Madex Plus. Its efficiency was evaluated not only in the laboratory, but also in field experiments in selected orchards (Jehle 2008; Kienzle et al. 2008). The virulence of other isolates genetically differing from CpGV-M was studied in the laboratory and in the field for their potential to overcome CpGV resistance (Eberle et al. 2008; Zingg 2008; Berling et al. 2009a, b). Furthermore, the molecular markers of resistance against CpGV-M of codling moths are intensively studied. According to the latest research (Asser-Kaiser et al. 2007), it can be assumed that the resistance is located on the sex chromosome Z (♂ZZ, ♀ZW). This conclusion was reached by single-pair crosses composed of one CpGV-sensitive (CpS) and one CpGV-resistant homozygotes (CpRR1), followed by a back-cross. The population created this way was later evaluated in bioassays (Asser-Kaiser et al. 2007).

According to our results, the population of codling moth from the plot treated for 12 years with CpGV-based biopesticides in Velké Bílovice expressed resistance to CpGV-M. Before using of CpGV-biopesticides, the population was treated for many years with insecticides. Then, cross-resistances to fenoxycarb, teflubenzuron, and phosalone was proved in Velké Bílovice population of codling moth (Stará, Kocourek 2007). Whereas cross-resistance between CpGV and some insecticides was not proved (Schmitt et al. 2008) we can expect a high efficacy of CpGV biopesticides against codling moth populations without history of CpGV treatment. These biopesticides can be suitable for organic apple orchards and in Integrated Pest Management (IPM) orchards with incidence of codling moth populations resistant to insecticides. Antiresistant strategies must be considered, however, as soon as these CpGV-based biopesticides are applied (Stará et al. 2009).

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