Evaluation of Efficacy of *Cydia pomonella granulovirus* (CpGV) to Control the Codling Moth (*Cydia pomonella* L., Lep.: *Tortricidae*) in Field Trials

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Abstract

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The efficacy of a CpGV-based preparation of Czech production against codling moth (CM) was tested in an experimental apple orchard at Prague-Ruzyně in 1998–2000. The influence of CpGV treatment on the reduction of CM population density and fruit injury was evaluated in comparison with teflubenzuron. Decline of CpGV efficacy in the orchard was tested in laboratory conditions on apples sampled at different terms after CpGV treatment. The rates of CpGV applied ranged from 0.5 to 1.00 × 10^{13} granulles/ha, and the number of applications from 3 to 5 per year. The biological efficacy of CpGV to reduce the CM population density ranged from 75.5% to 96.0%, that of teflubenzuron from 90.8% to 97.5%, compared to the untreated control. The CpGV treatment was more efficacious in reducing the CM population density than in reducing fruit injury. The efficacy of CpGV decreased to 50% after 20, 10 and 11 d after treatment in 1998, 1999 and 2000, respectively. According to our results, a 10 d interval for CpGV treatments is recommended in case of further mass egg-laying by CM in the period between CpGV applications.

Keywords: *Cydia pomonella*; *Cydia pomonella granulovirus;* apple orchards

*Cydia pomonella granulovirus (*CpGV) is a member of the genus *Granulovirus* (family *Baculoviridae*) (Crook 1991). It was first described in CM larvae collected from orchards of apple and pear in Mexico (Tanada 1994). After ingestion of CpGV by CM larva, the granulin dissolve in the alkaline midgut and release the virions that initiate infection in midgut epithelial cells and replicate and spread throughout the major body tissues, leading to death of the host (Federici 1997; Thiem 1997). CpGV is highly pathogenic for CM but harmless for nontarget organisms. Because of this characteristic, *Cydia pomonella granulovirus* CpGV-based microbial agents are frequently used in biological control of CM in apple orchards in the USA and Europe (Jaques 1994; Baudry *et al*. 1996; Biache *et al*. 2000; Hunter-Fujita *et al*. 1998).

Results of early field efficacy trials showed that applications of CpGV are comparable to applications of chemical insecticides in their ability to control CM damage to apples (Huber & Dickler 1977; Mantinger *et al*. 1992; Charmillot *et al*. 1991). The recommended rates of CpGV preparations range usually from 10^{11} granulles/ha to 5 × 10^{13} gran./ha (Sheppard & Stairs 1976; Audemard *et al*. 1992; Charmillot 1989, 1991). The number of applications of CpGV preparations depends mainly on the CM population density and number of CM

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generations at a locality (Huber & Dickler 1977; Jaques 1994; van der Geest & Evenhuis 1991). The short persistence of CpGV in the field necessitates applications in 7–14 d intervals (Polesný 2000; Audemard *et al*. 1992; Pasqualini *et al*. 1994).

CpGV is suitable for using in IPM and biological regimes of fruit production. However, certain conditions of CpGV application have to be fulfilled (Charmillot 1995; Baudry *et al*. 1996). Then, CpGV can be used in anti-resistant strategies, where the insertion of CpGV into the sequence of chemical pesticides can decrease the risk of resistance development. At present, preparations based on CpGV are registered in several European countries (Hunter-Fujita *et al*. 1998); in the Czech Republic registration is being prepared.

The aim of our study was to obtain information about the efficacy of a CpGV preparation from Czech production in conditions with high population density of CM and small size of treated plot. For better timing of treatments by CpGV-based preparations, the persistence of CpGV on apples after the treatment was tested in laboratory conditions by the introduction of CM larvae from a laboratory colony onto apples sampled in the orchard at different terms after treatment.

MATERIALS AND METHODS

The field experiments with CpGV based preparations were carried out in 1998–2000 in an experimental apple orchard at Prague-Ruzyně (50°06´N, 14°15´E, 364 m elev.). The applications of CpGV were made on a 0.34 ha plot on the apple varieties Melrose, Šampion, Denár and Rubín. The chemically treated plot of 0.34 ha (30 m distant from the CpGV treated plot) with the same varieties was treated in 1999 and 2000 by Nomolt 15 SC. As the untreated control an alley of apple trees of the variety Bernské růžové (0.1 ha, 450 m distant from the CpGV treated plot) and a plot with botanical apple varieties (0.2 ha, adjacent to both CpGV and chemically treated plots) were used.

The formulation of the preparations based on CpGV for the experiments was made in Chelčice. The CpGV strain was received from the BBA – Institute for Biological Control, Darmstadt, Germany, and was propagated on CM larvae from a Russian laboratory colony.

The CpGV based preparations were applied in 1998–2000 as sprays using a mist blower Hardi LE 465 and at a rate of 500 l/ha. The rates of CpGV were from 0.5 × 10^{13} gran./ha to 1.00 × 10^{13} gran./ha. The higher rate (1.00 × 10^{13} gran./ha) was used for the first treatment, and further applications received the lower rate. Nomolt 15 SC was applied in 1999 and 2000 at a rate of 0.08% using a mist blower Hardi LE 465. Applications of both CpGV preparation and Nomolt 15 SC were timed to coincide as closely as possible to periods of hatching of CM larvae. After the occurrence of a flight peak of CM males in pheromone traps the temperature conditions for egg laying were determined. When the temperature at 21:00 h reached 17°C during the period of flight peak, the calculating of DD 88°C above LDT 10°C for the embryonal development of eggs (Beránková 1984) started. The timing of sprays by calculating the DD for egg development was combined with a visual control of eggs in the orchard. Because of the short persistence of CpGV in nature, the applications were made in 10 d intervals. Terms of treatment with CpGV and Nomolt 15 SC are given in Table 1. Against the first generation three applications were made every year, against the second generation the number of applications ranged from zero (in 1999) to two (in 2000) (Table 1).

The efficacy of CpGV and Nomolt 15 SC to regulate the CM population was determined by counting the number of CM larvae in paper belt traps on treated as well as on untreated plots (Table 2) and by evaluation of injury to fruits (Table 3). The number of CM larvae in paper belt traps was checked during the whole season; the fruit injury was evaluated after the end of development of the 1st CM generation and at harvest.

The biological efficacy of CpGV and Nomolt 15 SC on the regulation of number of CM larvae in comparison with the untreated control was calculated by the formula

$$x_1 = 100 - [(100 \times y_1)/z_1]$$

where: x_1 – biological efficacy
y_1 – number of larvae/tree on the treated plot
z_1 – number of larvae/tree on the untreated plot

When fruit injury was evaluated, the proportions of shallow and deep entries in apples of the total number of counted fruits were calculated.

On the plot treated with CpGV, its persistence was determined on apples from the varieties Šampion and Denár. Fruits from an untreated variety from another part of the orchard were used as control.

Table 1. Terms of treatments by CpGV and Nomolt 15 SC and particular rates (Prague-Ruzyně, 1998–2000)

Year	Term of treatment	Rate of CpGV (gran./ha)	Rate of Nomolt 15 SC (%)
1998	3.6.	1×10^{13}	–
	9.6.	0.55×10^{13}	
	26.6.		
	27.7.		
1999	3.6.	1×10^{13}	0.08
	13.6.	0.66×10^{13}	0.08
	20.7.	0.575×10^{13}	0.08
2000	19.5.	1×10^{13}	0.08
	29.5.	0.5×10^{13}	0.08
	13.6.	0.5×10^{13}	0.08
	7.8.	0.5×10^{13}	0.08
	17.8.	0.5×10^{13}	0.08

Twenty fruits were taken from treated and untreated varieties before and immediately after CpGV treatment and then in weekly intervals. In the laboratory, L1 CM larvae from the laboratory colony were placed on fruits taken in the orchard. After two weeks incubation in the laboratory, the proportions of shallow and deep entries in fruits were calculated. The efficacy of CpGV to reduce deep entries in fruits in comparison with the untreated control was calculated by the formula

$$x_2 = 100 - [(100 \times y_2)/z_2]$$

where: x_2 – CpGV efficacy
y_2 – proportion of deep entries in apples treated by CpGV
z_2 – proportion of deep entries in untreated apples

The dependence between the number of days from CpGV treatment and the CpGV efficacy was quantified by linear regression (Table 4, Figure 2). The number of days after CpGV treatment when the CpGV efficacy had decreased by 50% was calculated from the linear regression formula (Table 4).

RESULTS

Changes in CM population density

The population density of CM on the plot used for the treatment with CpGV fluctuated from year to year. The average number of CM larvae in paper belt traps per tree ranged from 0.4 to 4 larvae (Table 2). The lowest average number per tree was recorded in 1998, the highest in 1999. In 2000, the average number of larvae in belt traps decreased to 2.1 larvae per tree. On the plot treated with Nomolt 15 SC, 1.5 CM larvae per tree were recorded in 1999 and only 0.3 in 2000 (Table 2). On the untreated plot with botanical varieties of apples, 9.8 larvae per tree were recorded in 1998. In 1999, that number increased to 16.3 and in 2000 decreased to 11.9 larvae (Table 2). On the untreated plot with the variety Bernské růžové only 1.5 larvae per tree were caught in 1998, followed by a rapid increase of CM population density in 1999, when the average number of CM larvae reached 36.8 larvae per tree. The high population density persisted in 2000 and 34.4 larvae per belt were recorded (Table 2).

The biological efficacy of CpGV to reduce the CM population density was 96%, 75.5% and 82.4% in 1998, 1999 and 2000, respectively. The biological efficacy of Nomolt 15 SC in reducing the CM population density was 90.8% in 1999 and 97.5% in 2000 (Table 2).

Fruit injury caused by CM

In 1998, the fruit injury on the CpGV treated plot after the end of development of the 1st CM generation was 4.2%, at harvest it had increased to 15.3% (Table 3, Figure 1). In 1999, the highest fruit injury was recorded on the plot treated with CpGV and on the one treated with Nomolt 15 SC. On the plot treated with CpGV, the fruit injury after

Table 2. Number of paper belt traps on the experimental plots, the average number of CM larvae per tree and biological efficacy of CpGV and Nomolt 15 SC treatment in reducing the CM population density (Prague-Ruzyně, 1998–2000)

Year	Plot	Number of paper belt traps	Ø number of larvae per tree	Biological efficacy of treatment (%)
1997	untreated plot (treated from 1998 on with CpGV)	20	6.1	–
	untreated control (botanical var.)	4	25.0	–
1998	CpGV	40	0.4	96
	untreated control (botanical var.)	5	9.8	–
	untreated control (var. Bernské růžové)	4	1.5	–
1999	CpGV	50	4	75.5
	Nomolt 15 SC	50	1.5	90.8
	untreated control (botanical var.)	4	16.3	–
	untreated control (var. Bernské růžové)	5	36.8	–
2000	CpGV	75	2.1	82.4
	Nomolt 15 SC	75	0.3	97.5
	untreated control (botanical var.)	10	11.9	–
	untreated control (var. Bernské růžové)	5	34.4	–

the 1st CM generation was 12.9% and at harvest it had increased to 28.5%. On the plot treated with Nomolt 15 SC, the fruit injury was 3.9% after the 1st CM generation and reached 15.4% at harvest (Table 3, Figure 1). In contrast to 1999, the lowest fruit injury was recorded on both treated plots in the following year 2000. On the plot treated with CpGV, the fruit injury was 7.8% after the end of development of the 1st CM generation and 10.1% at harvest. On the plot treated with Nomolt 15 SC, the fruit injury was 2.5% after the 1st CM generation and and 4.0% at harvest. On the other hand, fruit injury on the untreated plot was 32.5% after the 1st CM generation and 28.6% at harvest (Table 3, Figure 1).

When fruit injury was evaluated, considerable differences in the proportions of shallow and deep entries in fruits on the plots treated with CpGV

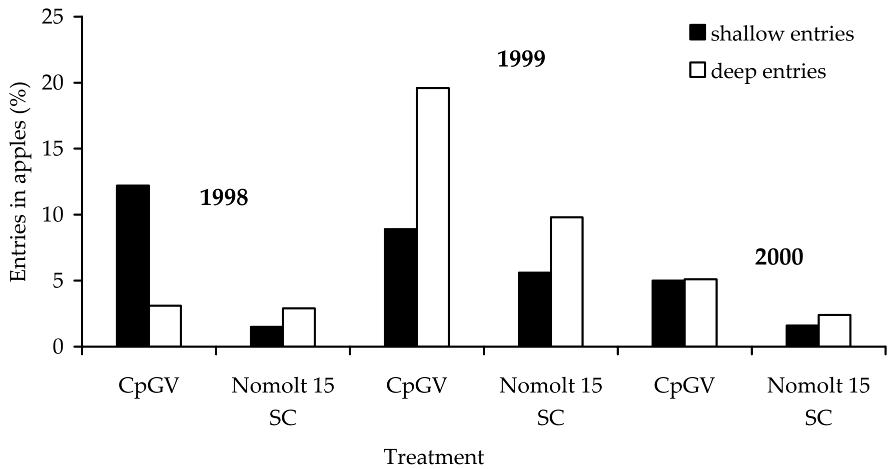

Figure 1. Fruit injury (evaluated at harvest) caused by codling moth larvae after treatment by CpGV preparation and Nomolt 15 SC (Prague-Ruzyně, 1998–2000)

Table 3. Fruit injury caused by codling moth evaluated after the development of the 1st CM generation had ended and at harvest (Prague-Ruzyně, 1997–2000)

Year	Plot	Fruit injury after the 1st CM generation had developed				Fruit injury at harvest			
		number of fruits	% SE	% DE	% total	number of fruits	% SE	% DE	% total
1998	CpGV	2658	3.1	1.1	4.2	1590	12.2	3.1	15.3
1999	CpGV	900	4.2	8.7	12.9	1166	8.9	19.6	28.5
	Nomolt 15 SC	700	0.7	3.2	3.9	483	5.6	9.8	15.4
2000	CpGV	1000	3.4	4.4	7.8	1000	5.0	5.1	10.1
	Nomolt 15 SC	1000	1.1	1.4	2.5	1000	1.6	2.4	4.0
	Untreated (var. Bernské růžové)	100	5.0	27.5	32.5	100	3.2	25.4	28.6

% SE = proportion of fruits with shallow entries (1–5 mm); % DE = proportion of fruits with deep entries (> 5 mm); % total = total proportion of fruits with entries

and with Nomolt 15 SC were found. On the plot treated with CpGV, the proportion of shallow entries ranged from 3.1% to 4.2% after the 1st CM generation and from 5.0% to 12.2% at harvest (Table 3). On the plot treated with Nomolt 15 SC the proportion of deep entries ranged from 0.7% to 1.1% after the 1st CM generation and from 1.5% to 5.6% at harvest (Table 3). On average, the proportion of shallow entries was 6.1% on the CpGV treated plot, while on the plot treated with Nomolt 15 SC it was only 2.1% (Table 3). The proportion of shallow entries was higher on the plot treated with CpGV than on the chemically treated plot (F = 3.345, P = 0.054).

Laboratory evaluation of CpGV persistence on fruits treated with CpGV

During the 3 years of CpGV treatment, the same trend in the frequency of shallow and deep entries after CpGV application and in the period betwen applications was found. One day after CpGV treatment the proportion of deep entries decreased considerably while that of shallow entries increased.

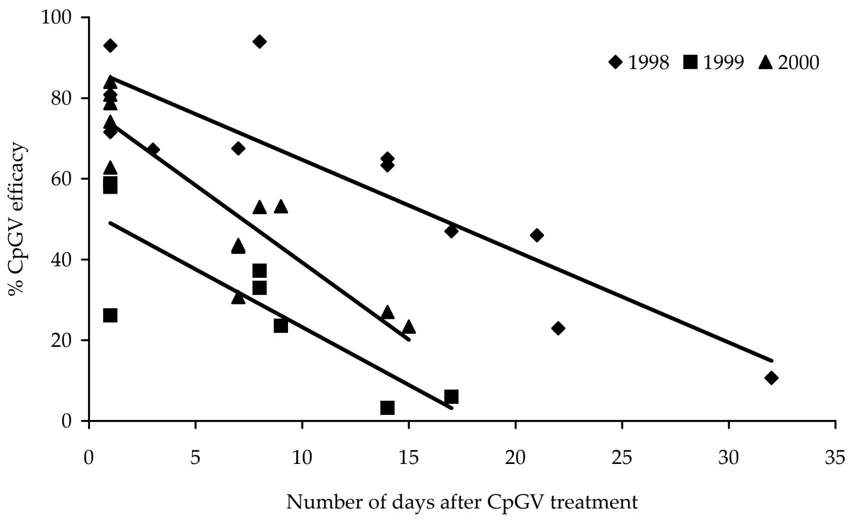

Figure 2. Decrease of CpGV efficacy to reduce deep entries in apples in dependence on number of days after CpGV treatment; Prague-Ruzyně 1998–2000. The course of decrease of CpGV efficacy is quantified by linear regression

Table 4. Decrease of CpGV efficacy to reduce deep entries in fruits in dependence on the number of days after CpGV treatment quantified by linear regression

Year	Linear regression formula	R^2	Number of days after CpGV treatment when CpGV efficacy decreased by 50%
1998	$y_2 = -2.2636x_2 + 87.362$	0.7909	20.5
1999	$y_2 = -2.864x_2 + 51.874$	0.7112	9.8
2000	$y_2 = -3.8217x_2 + 77.482$	0.8112	10.6

Table 5. The proportion of deep entries in apples in dependence on number of days after CpGV treatment (Prague-Ruzyně, 1998–2000)

1998			1999			2000		
Number of days after CpGV treatment	proportion of deep entries in apples (%)		number of days after CpGV treatment	proportion of deep entries in apples (%)		number of days after CpGV treatment	proportion of deep entries in apples (%)	
	CpGV	untreated control		CpGV	untreated control		CpGV	untreated control
1	36	100	1	42	100	1	20	94
6	40	73	8	67	100	8	47	100
1	27	95	1	35	85	1	23	89
3	21	64	9	68	89	7	53	94
17	53	100	17	94	100	14	73	100
1	18	94	1	65	88	1	35	94
8	6	100	8	59	94	7	61	88
14	33	90	14	60	62	15	72	94
22	77	100				1	15	94
32	75	84				9	44	94
1	7	100				1	18	94
7	25	77				7	50	88
14	35	100						
21	47	84						

CpGV = apples from the plot treated with CpGV; untreated control = apples from the untreated plot

In the period between the CpGV applications the proportion of deep entries gradually increased (Table 5).

The longest persistence of CpGV on apples was recorded in 1998, the shortest in 1999. The decrease of CpGV efficacy on apples treated with the CpGV-based preparation was expressed by linear regression (Table 4). In 1998, a decrease of CpGV efficacy to 50% was recorded 20.5 d after CpGV application, while in 1999 it occurred after 9.8 d and in 2000 after 10.6 d (Table 4).

DISCUSSION

The influence of CpGV treatment on changes in CM population density

In the experimental apple orchard at Prague-Ruzyně no chemical treatment against CM had been practised before 1998. Consequently, there was a very high population density of CM on the experimental plots when the experiments with CpGV started (Table 2). On both untreated plots the high population density continued during the experiments and fluctuated only slightly between years. On the plot treated with CpGV the population density fluctuated similarly to the untreated plots, with the highest average catch of CM larvae per tree in 1999. On the chemically treated plot, the population density decreased considerably after the treatment with Nomolt 15 SC.

The influence of CpGV treatment on the level of fruit injury caused by CM

The fruit injury caused by CM larvae in 1998–2000 was high and exceeded the damage threshold on both CpGV and chemically treated plots. The average fruit injury on the plot treated with CpGV was approximately double of that on the chemically treated plot. In accordance with Glen and Payne (1984) we found higher CpGV efficacy in reducing the number of CM larvae in paper belt traps than in reducing the fruit injury.

In the first year of CpGV treatment the fruit injury was probably higher because of the high initial population density of CM on the plot. In 1999, high fruit injury was recorded on both CpGV and chemically treated plots. It was caused by several circumstances. First, the whole CM population was concentrated on a low number of fruits due to the low set of fruits in that year. Second, the first CpGV application against the 1st CM generation was late and thus did not affect all hatched CM larvae. In addition, UV light caused further degradation of CpGV. Further, absence of the treatment against the second CM generation also resulted in higher fruit injury at harvest. This confirmed the necessity to control both generations. In 2000, the lowest fruit injury was found on both treated plots. On the plot treated with CpGV the fruit injury at harvest was 10%, while that on the plot treated with Nomolt 15 SC was only 4%. Additionally, the lower number of CM larvae in paper belt traps attested to the higher efficacy of the used treatment. This higher efficacy was probably influenced also by favourable weather conditions, mainly because of low rainfall in the period of the first two applications of CpGV against the 1st CM generation. These applications are the most important in determining the level of the CM population.

Both CpGV and chemically treated plots were insufficiently isolated from other parts of the orchard, which influenced the efficacy of treatment. On the untreated plot next to both treated plots a high CM population density was recorded during the years of the experiment. The untreated plot thus served as a source of CM females that could migrate to treated plots and influence the efficacy of any treatment used there.

The small size of the treated plots also influenced the level of fruit injury. A comparison with the results of experiments carried out in an intensive apple orchard at Velké Bílovice (Pultar – unpubl.) may be opportune here. During 1997–2000, the same CpGV preparations as at Prague-Ruzyně were tested at Velké Bílovice on 2.09 ha and 1.33 ha plots. Although the population density of CM was higher at Velké Bílovice than at Prague-Ruzyně, the efficacy of CpGV was comparable to chemical treatment. In 1998 at Velké Bílovice, CpGV was more efficient than *Bacillus thuringiensis* (Biobit XL) and its combination with chlorpyrifos-methyl (Reldan 40 EC). Four years of CpGV treatment by direct spraying at Velké Bílovice lowered fruit injury at harvest to 3–7% with a trend of further decline and stagnation at a level of 2–4% (Pultar 1999 – unpubl.).

When the fruit injury was evaluated, a higher proportion of shallow entries was found in fruits of the CpGV plot than in those of the chemically treated plot. The proportion of shallow entries increased significantly after CpGV treatment in comparison to the proportion of shallow entries after chemical treatment. The potential of CpGV application to increase the incidence of shallow entries in fruits was confirmed by Jaques (1994). The increased level of shallow entries reflects the effect of CpGV on CM. The virus kills the larvae slowly, allowing them to still cause shallow injuries (Falcon *et al*. 1968). However, if these injuries are caused by larvae of the 1st CM generation, they can heal up till harvest (Charmillot 1995; Huber & Dickler 1977).

Frequency of CpGV treatment against CM in dependence on the CpGV persistence on apples

The CpGV persistence on apples in the field differed according to conditions of a given year. During 1998–2000 a different length of CpGV persistence on fruits was recorded. It was longest in 1998, and shortest in 1999. The decrease in CpGV efficacy to 50% was recorded approximately after 10 (1999), 11 (2000) or 20 (1998) d after treatment. From our results we suppose a maximal interval between CpGV applications of 10 d. When 10 d after treatment the next mass egg hatching occurs, it is necessary to repeat the application. This confirms the results of previous studies (Polesný 2000;

Audemard *et al*. 1992; Pasqualini *et al*. 1994) that recommended an interval of 7–14 d between CpGV applications. In contrast, Glen and Payne (1984) found that CpGV activity declined to 50% after 3 d from application. Fast inactivation of CpGV by UV light was confirmed by Charmillot *et al*. (1998). They pointed out that CpGV activity declined to one half 5–8 d after application. Accordingly, sunny weather following the CpGV application can rapidly lower its efficacy.

The results of 3 years experiments with CpGV proved that several preconditions have to be met to ensure high efficacy of this method of protection against CM. They are mainly: sufficient isolation from neighboring parts of an orchard with high CM population density, and then an adequate size of the treated plot. This confirms the results of other authors (Falcon *et al*. 1968; Huber & Dickler 1977; Charmillot 1995). Also, low initial CM population density is important for a high efficacy of CpGV treatment (Audemard *et al*. 1992; Baudry *et al*. 1996). Pasquier and Charmillot (1998) pointed out that at low initial population density of CM (one CM larva per tree) it is possible to maintain the pest population at a low level for a long time. In orchards with high CM population density, it would first be necessary to reduce the CM population density by conventional insecticides and then start with CpGV treatment to ensure high efficacy of CpGV treatment against CM. It is also possible to include CpGV treatment in the sequence of conventional insecticides to avoid the development of resistance by CM.

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Souhrn

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Účinnost CpGV proti obaleči jablečnému byla testována v letech 1998–2000 v experimentálním sadu v Praze-Ruzyni. Byl vyhodnocen vliv přípravku na bázi CpGV české provenience na změny populační hustoty obaleče a na stupeň poškození plodů v porovnání s teflubenzuronem. Persistence CpGV v sadu byla testována v laboratoři na plodech odebíraných v sadu v různých termínech po ošetření CpGV. Preparát na bázi CpGV byl aplikován v dávkách CpGV od $0,5 \times 10^{13}$ do $1,00 \times 10^{13}$ gran./ha, počet postřiků se pohyboval od 3 do 5 za rok. Biologická účinnost CpGV na redukci populační hustoty obaleče se pohybovala od 75,5 % do 96 %, biologická účinnost teflubenzuronu se pohybovala od 90,8% do 97,5% v porovnání s neošetřovanou kontrolou. Účinnost CpGV byla vyšší na redukci populační hustoty obaleče jablečného než na poškození plodů. Pokles účinnosti CpGV na 50 % byl v letech 1998–2000 zjištěn po 20, 10 a 11 dnech po ošetření. Na základě těchto výsledků byl stanoven doporučený interval pro opakování ošetření CpGV 10 dní v případě dalšího kladení vajíček obaleče v období mezi postřiky.

Klíčová slova: *Cydia pomonella*; *Cydia pomonella granulovirus*; jablečné sady

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