

THE EFFICIENCY OF BIOLOGICAL CONTROL TREATMENTS OF CODLING MOTH (*CYDIA POMONELLA* L.) ON THREE DIFFERENT APPLE VARIETIES

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Abstract. Codling moth (*Cydia pomonella* L.) is one of the most important apple pests which population is steadily growing. The aim of this study, conducted in Eastern Croatia, was to determine the efficiency of two biological preparations CpGV and kaolin clay compared with pyrethroids and control treatment. The preparations used in treatments were: 1) *Cydia pomonella* granulovirus (CpGV), 2) deltamethrin, beta cyfluthrin and alpha-cipemetrina, 3) kaolin, 4) control treatment, applying in two different methods of time control, on the three apple varieties ‘Melrose’, ‘Jonagored’ and ‘Golden Delicious’ clone B. The efficiency of the treatment 1 (CpGV) ranged from 78% to 95%, of treatment 2 (pyrethroids) from 96% to 97%, and treatment 3 (kaolin) – between 21.5 and 57%. There was significant negative correlation between yield and damage ($r = -0.56$). Two methods of determining time control did not differ significantly. Variety ‘Melrose’ had the highest number of fruits damage in all three years and ‘Golden Delicious’ clon B the lowest number which can be related to the codling moth caterpillar affinity to a certain variety. It is very important to make the best choice of preparations in control and at the same time be sure of their positive effect on the environment and human health.

Keywords: *CpGV, kaolin, pyrethroid, time of control, variety*

Introduction

The apple is a fruit species with the highest number of control treatments in the world (Blommers, 1994). In Croatia, the total number of apple control treatments are around 15 and 25 against all diseases and pests, while the number of control treatment of codling moth ranges from 6 to 8 treatments during one growing season. Despite ecological concern about the harmful pesticides effects on the environment, control of phytophagous species is mostly based on the use of chemical control (Choinard et al., 2016; Iraqui and Hmimina, 2016; Tamošiūnas et al., 2014). In the orchard with use of insecticides with

broad spectrum of action, the result is absence of useful entomofauna diversity (Ismail and Albittar, 2016; Blommers et al., 1987) which contributes to the reduction of natural phytogamous population with no harmful effects on the environment. Biological control incorporates various approaches called integrated pest management which combines a variety of pest control methods (Mahr et al., 2008). The biological control methods imply use of pheromones and mating disruption (Ciglar, 1998; Maceljski, 2002), natural predators and parasitoids (Lacey and Unruh, 2005) as well as use of biological control agents (CpGV - *Cydia pomonella* granulovirus and EpNS - entomopathogenic nematodes). *Cydia pomonella* granulovirus (CpGV) was used for 30 years in the most European countries (Sauer et al., 2017) while in Croatia it was registered in 2011 when its commercial use started. Microbial agent (CpGV) belongs to genus *Granulovirus*, family *Baculoviridae* (Crook, 1991). It is highly pathogenic for codling moth and for some related moth species (Luque et al., 2001) but harmless for nontarget organisms (Stara and Kocourek, 2003).

The research hypothesis was that biological preparation on the basis of baculovirus would provide satisfactory fruit quality (codling moth damage less than 1%) as well as more ecological acceptance than chemical preparations (pyrethroid treatment) while kaolin treatment would have less fruit damage than the control treatment. The second hypothesis was that monitoring of codling moth life stage in insect cage would be more accurate in determining the time control than the flight monitoring on pheromone traps. The third hypothesis was the existence of codling moth affinity according to certain varieties. According to hypothesis the first aim of this study was to examine the efficiency of biological products in Eastern Croatia on the basis of CpGV and kaolin clay as well as the efficiency of plant protection products from the group of synthetic pyrethroids compared with control treatment. The efficiency of treatment is manifested through two characteristics: fruit damage from codling moth (% damage), which is a direct indicator, and fruit yield. The second aim of this research was to determine the time of application using two methods: monitoring developmental codling moth stages in insect cage and using pheromone traps for codling moth and summing the effective temperatures. The third aim was to determine the affinity of codling moth to the tested varieties.

Materials and methods

Study sites

The experiment was performed at the orchard of Agricultural Institute Osijek, Eastern Croatia (45°31'44,56" N, 18°45' 44,88" E) during 2012–2014 in four different control treatments of codling moth (*Cydia pomonella* L.). In 2012 climatic conditions were less favourable for the apple production, because of the period of negative temperatures down to –6 °C during the phenological stage of development BBCH 5–59 when apple was in the stage of floral balloons. Low temperatures caused a decrease in the apple yield for 50% in 2012. During 2013 and 2014 there were not similar extremes that could have a negative effect on the apple production. Climate data for this research was collected by weather stations which were located in the experimental orchard (Figs. 1, 2 and 3).

The treatments

Treatment 1 was based on biological product baculovirus (Granupom), used in a dose of 150 ml/ha/1 m height, with the addition of 300 g sugar. Treatment 2 was based

on the group of synthetic pyrethroids- beta-ciflutrin (Beta Baythroid EC 025) in a dose of 500 ml ha⁻¹, deltametrin (Rotor 1.25 EC) in dose of 600 ml ha⁻¹ and alfa-cipemetrin (Fastac 10 SC) in dose of 250 ml ha⁻¹. Chemical preparations from group of synthetic pyrethroids were involved in this research because of expected good results in efficiency in codling moth control as well as comparisons with the efficiency of biological products. Treatment 3 was based on the kaolin clay (Cutisan) 15 kg ha⁻¹ according to manufacturer's recommendations for codling moth control. Treatment 4 was control or untreated plot. The experiment included three apple varieties 'Golden Delicious' clone B, 'Jonagored' and 'Melrose'. Growing form of trees was spindly bush. Experimental orchard was planted in 2005 on the vegetative rootstock MM106. Experimental orchard was on 0.4 ha and divided into four equal parts, which represents different codling moths control treatments. Each part, from each other was separated with protective zone of three trees. Experimental orchard was also divided through the whole length in two equal parts, left and right side with 60 trees in each side. Every side of the same control treatment represents different method of time control. Every block of four experimental parts in orchard contained 5 experimental trees in the row of each variety, in total there were three rows with all three varieties with 15 trees per time of control, 30 trees per treatment, 120 trees in all four treatments. In the left part of experimental orchard (which contained 60 experimental fruit trees per one method of time control), treatments were done on the basis of monitoring imago flight by pheromone traps (critical number (threshold) was 3–5 adults per trap in one day or in several days) and after catching critical number per trap started with summarizing effective temperature till sum of 90 °C (which represents time of hatching larvae-L1. In the other part of the experimental orchard (which contained another 60 experimental fruit trees), the treatments were done on the basis of controlling the pest biology in insect cage for first generation combined with Wildbolz method (Wildbolz, 1962) for other generations. This method is based on the regular summarizing the effective temperature starting from January 1. Caterpillar hatching started at sum 90 °C of effective temperatures. At the 100 °C of effective temperatures codling moth flight started. The effective sum of 610 °C is necessary for ending period of one codling moth generation. During the fruit harvest every experimental tree was harvested separately, fruits were placed in labelled boxes and 100 apples were randomly allocated from each tree according to EPPO standards (2004) for visual control of damage. A 1500 apples were examined per time of control on three varieties, 3000 fruits were examined per treatment in left and in right side of experimental orchard which represents different time of control. In total there were examined 12000 apples in all four treatments and in two different methods of time control. After visual inspection of 100 apples the damage based on the number of incoming and outgoing tunnels and wholes from caterpillars of codling moth was determined.

Statistical analysis

Statistical analysis was performed using R software (R core team, 2013). *Anova* function from *car* package was used for factorial analysis of variance with four sources of variation: time of control, codling moth treatment, variety and replication. *Agricolae* package was used to calculate Tukey's honest significant difference (HSD) test at $P < 0.05$ level (HSD_{0.05}).

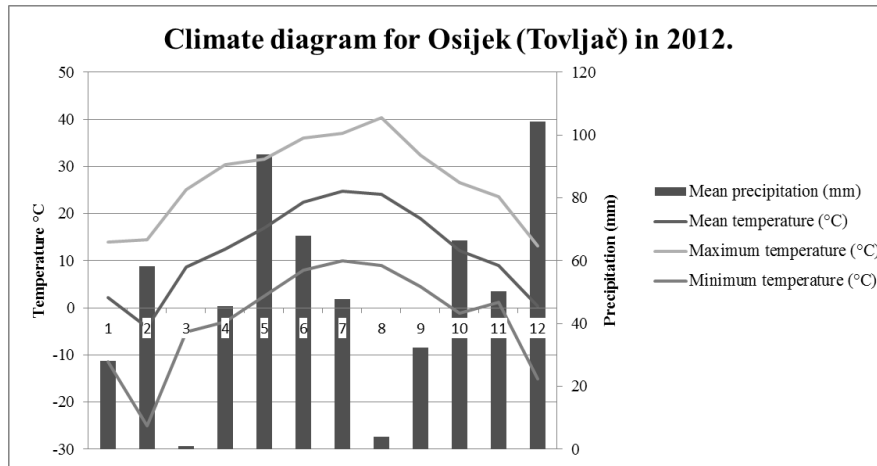


Figure 1. The monthly meteorological data for experimental orchard during 2012

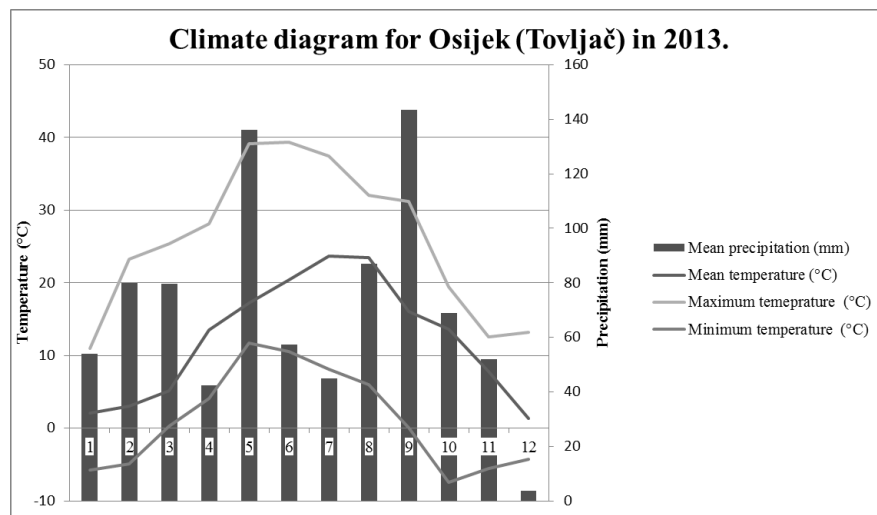


Figure 2. The monthly meteorological data for experimental orchard during 2013

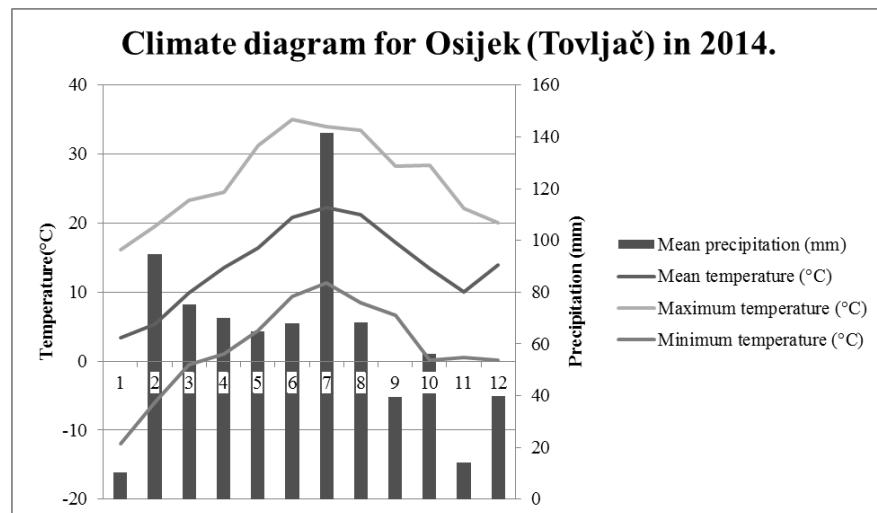


Figure 3. The monthly meteorological data for experimental orchard during 2014

Results

Analysis of variance (ANOVA) revealed consistent non-significant effects of replication, time of control and interactions with time of control over the three years for both traits (Table 1). The effects of codling moth treatment and variety were mostly highly significant. The only significant interaction in the analysis was detected between codling moth treatment and variety for yield in 2013.

Table 1. Results of F-test from ANOVA for fruit yield and damage caused by codling moth (transformed data) in a three-factor design experiment conducted in Eastern Croatia over three years

Source of variation	df	Yield (kg)			Damage (%-transformed)		
		2012	2013	2014	2012	2013	2014
Replication (R)	4	1.2	2.6	0.2	1.1	0.6	0.2
Time of control (T)	1	0.1	2.8	0.01	0.6	1.5	3.0
Codling moth treatment (C)	3	39.1**	3.4*	22.0**	574.9**	1118.0**	318.6**
Variety (V)	2	4.0*	6.2**	43.5**	5.8**	5.5**	5.7**
T × C	3	0.7	1.5	2.1	0.01	0.04	0.1
T × V	2	0.3	2.3	0.1	0.04	0.01	0.4
C × V	6	1.4	3.9**	1.8	1.5	1.2	1.3
T × C × V	6	0.8	1.4	0.6	0.1	0.04	0.5

df – degrees of freedom, ** – significant at the 0.05 and 0.01 probability levels, respectively

The separate analysis over three years are shown in Figure 4. demonstrating different mean values for fruit yield and damage across the codling moth treatment and variety. The pyrethroid treatment performed consistently the best results in comparison with baculovirus treatment and kaolin in all three tested years. Moreover, the differences between pyrethroid and baculovirus treatments were significant in all three varieties in 2012 and 2014. The highest yield (71.35 kg) in 2013 and the lowest percentage of fruit damage (1%) had variety ‘Golden Delicious’ clone B in the same treatment.

Combined ANOVA showed that fruit yield and damage differed significantly among treatments in all three years. The interactions between three codling moth damages and yield were significant in all three years of investigation. There were negative relations between the two traits with different association strengths across the three years (Fig. 5). The strongest association was in 2012 when the highest mean values for damage and the lowest mean values for yield were observed.

In the three-year field experiment, the highest average efficiency had treatment 2 with pyrethroids (96.6%), followed by treatment 1 with CpGV (85.86%) and the lowest efficiency had treatment 3 with kaolin (45.16%). The efficiency of pyrethroids was stable across the years (Table 2).

Discussion

Difference in yield between varieties among different treatments was expected because fruitfulness is not only result of intensity of pest infestation but genetic characteristics of each variety among other different but very important factors, such as

fertilization, growth form, pruning, climate condition. In 2012, average yield of all three varieties were 19.11 kg which can be explained by the influence of frost and low temperatures during beginning of apple flowering in this year (Fig. 4).

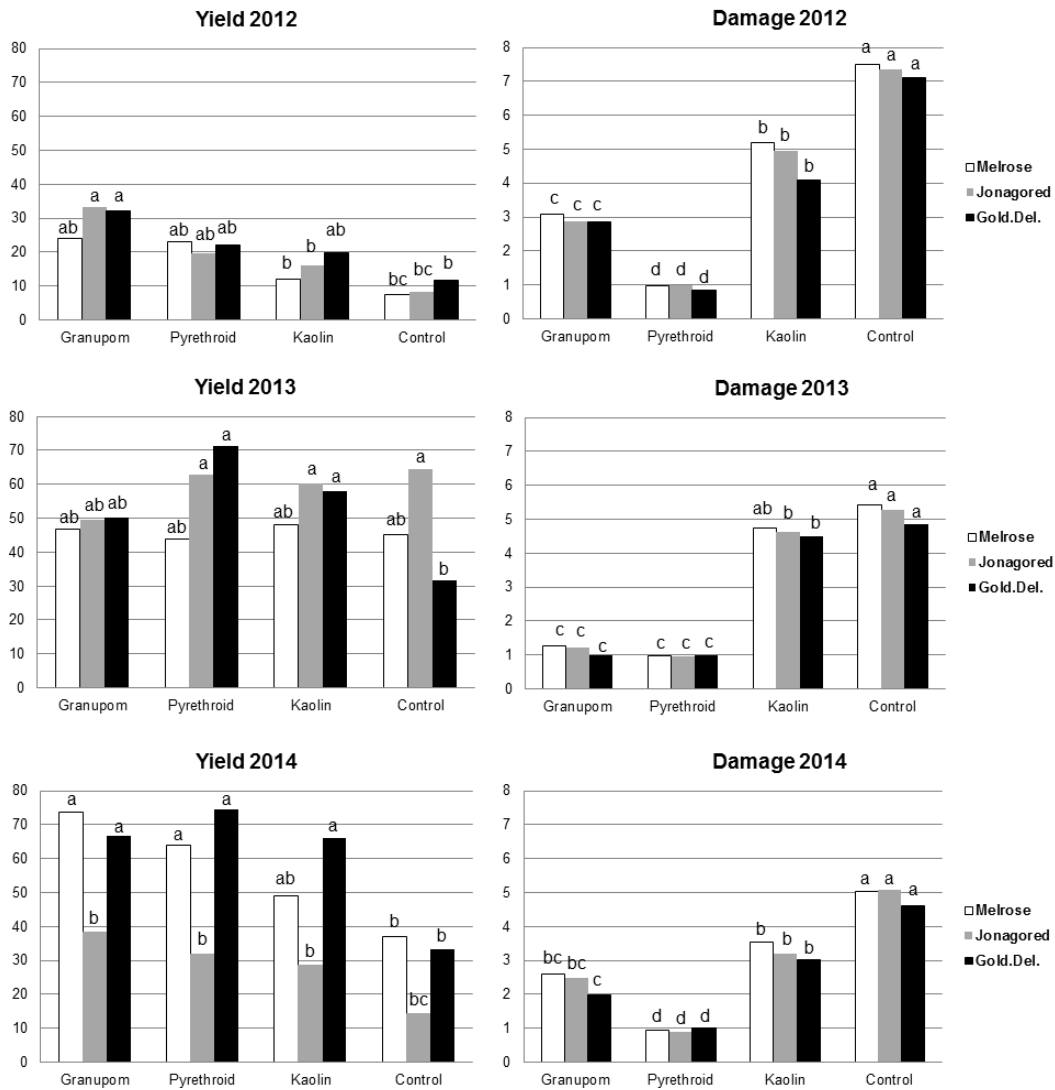


Figure 4. Mean values for fruit yield (kg) and fruit damage (%-transformed) in three apple varieties across three codling moth treatments and control averaged over two methods of time control of codling moth. Means with the same letters are not significantly different according to the Tukey's $HSD_{0.05}$

Table 2. Efficiency of applied treatments in the control of codling moth in 2012, 2013 and 2014 at locality Agricultural institute Osijek in Eastern Croatia

Treatment	Efficiency %			
	2012	2013	2014	Average
1 (CpGV)	84.60	95	78	85.86
2 (pyrethroids)	97	97	96	96.6
3 (kaolin)	57	21.5	57	45.16
4 (control)	—	—	—	—

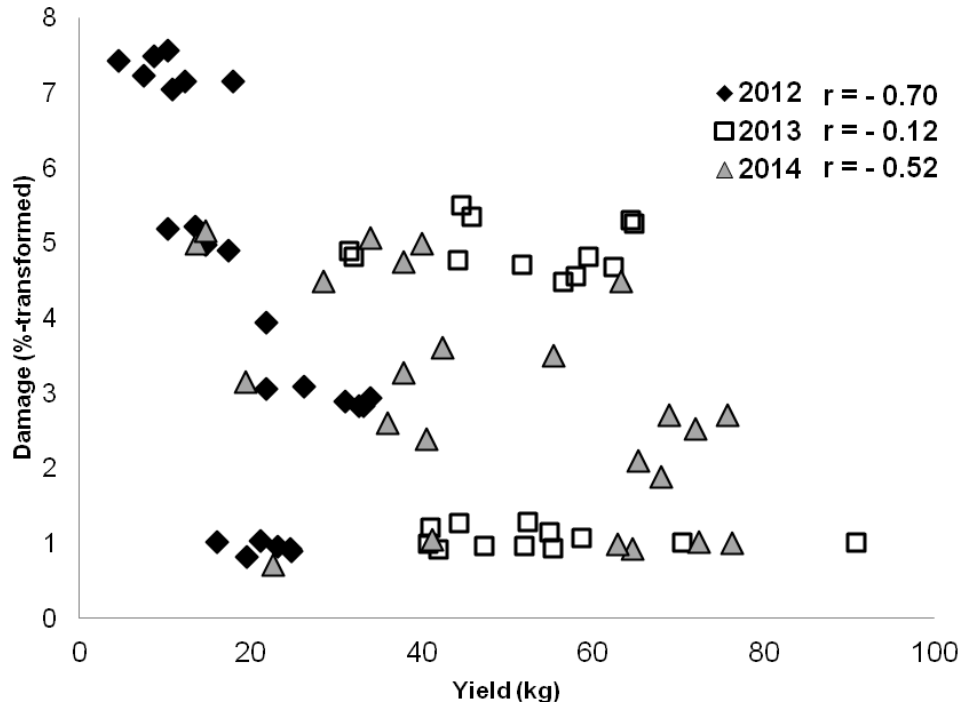


Figure 5. Relations between fruit yield and codling moth damage across the three years including all times of control, moth treatments and varieties. Note: Correlation coefficients (r) between the traits are indicated for specific year

A research in America showed that differences in annual fruit yield among three different production systems were inconsistent (Reganold et al., 2001). In this research damage on the apples from the codling moth was different between treatments and in the years of study. The higher percentage (more than 1%) of fruit damage in treatment 1 (CpGV), occurred due to short period of residual control which requires more frequent applications because of its sensitivity to solar radiation and its incapability to be exposure to UV radiation and extreme high temperatures (Arthurs and Lacey, 2004; Lacey and Arthurs, 2005). Baculovirus can persist for long periods in water and in the soil when is protected from sunlight and extreme high temperatures (Hajek et al., 2017). According to Wearing (1993), this virus has slow action and less efficiency in preventing codling moth damage. Results of early field efficiency trials showed that applications of CpGV are comparable to applications of chemical insecticides in their ability to control codling moth apple damage (Huber and Dickler, 1977; Mantinger et al., 1992). The best results with lowest percent of apple damage were in treatment 2 (pyrethroids). Conventional production in apple orchard results with little damage at harvest period (Suckling et al., 1999) but frequent insecticide applications are harmful to human health, the environment and beneficial organisms (Pajač et al., 2011). The most similar results in fruit damage with control had treatment 3 which included use of kaolin clay as shown in Fig. 5. According to Unruh et al. (2000), reduced number of codling moth damage was noticed on the apple fruits with kaolin film in compare with the control results. Kaolin clay particles do not kill pests but repellent them or act as barrier (Glenn, 2016; Glenn et al., 2002; Showler and Sétamou, 2004; Lapointe, 2000). Unruh et al. (2000) stressed that film of kaolin clay particles on fruits are very good protection against pest but only when their population is very low.

In this study, a highly significant negative correlation between yield and fruit damage was recorded. A study in the US also showed that fruit damage of codling moth was negatively correlated with yields so that in years of low crop damage percentage was relatively high, while in years of high yield percentage of damage was relatively low (Clark and Gage, 1997). This occurred because the same population of codling moth, in the years of smaller yield, had a smaller amount of available food. Combined analysis of variance showed highly significant differences between the years of research, considering the yield and apple fruit damage from codling moth. Less significance occurred in the source variation treatment \times time of control \times variety. This indicates that climate has great impact on the yield and fruit damage, and that the year is the most critical factor in production of apples. In the same conditions of apple production, using the same treatments, time of control and varieties, characteristic of yield has very low repeatability. Correlation between yield and fruit damage was negative and significant ($P < 0.01$), and increasing damage on the apples from codling moth reduces yield.

Results of conducted research in treatment 1 (CpGV) were similar to the results of efficiency from Czech Republic which varied from 75.5 to 96% (Stara and Kocourek, 2003). In Germany, the efficiency of CpGV was 70% (Kienzle et al., 2002). The reason for reduced efficiency could be because of use CpGV preparations for many years (Arthurs et al., 2004).

Treatment 2 (pyrethroids) efficiency varied from 96 to 97% while results of cypermethrin efficiency from Serbia varied from 92.2 to 97.6% (Miletić et al., 2011). Pyrethroids still have good efficiency but they also have broad spectrum of impact on beneficial insect so it is reason of limited number of applications during year season in integrated production.

The efficiency of treatment 3 (kaolin clay), varied between 21.5 and 57%. The average efficiency in three years amounted to 45.16%. This preparation is primarily used in orchards as protection from strong direct sunlight and to prevent the fruit surface sunburn (Glenn et al., 2002). In this study was shown that kaolin have effect on minimizing damage on the fruits from codling moth in comparison to control as confirmed by other studies (Showler, 2003; Knight et al., 2000; Unruh et al., 2000). Due to a low efficiency below 60%, and its main effect in the prevention sunburns, should be included in the program of controlling first generation of codling moth (Knight et al., 2000) as well as additional product in an integrated control production. Statistical analysis did not confirm significant difference among different methods of calculating time control of *Cydia pomonella* and yield as well as fruit damage. It seems that both methods of calculating time control are based on monitoring of codling moth biology getting therefore equal results. The different number of fruit damages between varieties within the same treatment gives the conclusion of bigger or less attraction of codling moth caterpillar for certain variety. The similar conclusion was made according to other authors (Hussain et al., 2014; Wenninger and Landolt, 2011; Landolt and Guédot, 2008). Biological control can be effective, economical and safe and it should be more widely used than it is today (Mahret et al., 2008).

Conclusions

1. The *Cydia pomonella* granulovirus (CpGV) based preparation had a lower average efficiency, 88.86%, compared to the preparations from the group of synthetic pyrethroids and was with 4.88% average fruit damage in the treatment. The kaolin

preparation had an average efficiency of 45.16% with 17.99% average fruit damage. Both preparations should be used in codling moth control program because of their positive impact on environment and human health. 2. Efficiency of two methods of determining control time did not differ significantly. Both methods showed equally good results.

3. Varieties in the study showed statistically significant differences in yield and fruit damage in all years of study. 'Melrose' had the highest number of fruits damage in all three years which can be related to the codling moth caterpillar affinity to a certain variety, than came 'Jonagored' and least attracti was 'Golden Delicious' clone B. It can be concluded that the affinity of codling moth to apple fruit is stronger when fruit surface has dominant red coloration and in this case further investigation is required.

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