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Introduction of Mating Disruption Pheromones in apple orchards in Fyr of Macedonia

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Abstract: During the 2014 and 2015 seasons, the control of codling moth (CM), *Cydia pomonella* (L.), in apple orchards in Prespa Region in Fyr of Macedonia was conducted with mating disruption pheromones. The apple is a dominant crop in this region and the codling moth pressure is constantly very high. Mating disruption technique in apple orchard was utilized for the first time with a single application of Isomate C TT dispensers per season. Population density of codling moth and fruit damage at harvest were kept at a low level. At the same time, population density of the pest and fruit damage in the conventionally treated orchards were higher and was serving as a reference. Based on our results, Isomate C TT dispensers may represent a promising alternative to traditional programs trying to control high initial infestation by codling moth and should be involved in the Integrated Pest Management practices on apple crops.

Key words: apple, codling-moth, *Cydia pomonella*, mating-disruption, Isomate C TT, fruit damage

Introduction

Codling moth (CM) *Cydia pomonella*, is a serious pest on pome fruit and walnut with preference to apple and if not properly controlled, it causes heavy losses both in Fyr of Macedonia and abroad. The pest was originally present only in Eurasia, but currently it can be found causing economic losses in North and South America, South Africa, Australia and New Zealand (Franck *et al.*, 2007 in Pajic *et al.*, 2011). The ways to control CM have been changing throughout the history from chemical to biological means of protection (Pajic *et al.*, 2011). However, despite numerous chemical treatments applied in some commercial apple orchards, fruit damage rate is still very high. In order to obtain fruits without any damage, there is a need to apply at least four treatments against CM in Fyr of Macedonia (Postolovski & Lazarevska, 1994), more than four treatments in Serbia (Miletic *et al.*, 2011), six or more chemical treatments per season in commercial orchards in Poland (Pluciennik, 2013), and eleven to fifteen treatments in Bulgaria (Kutinkova *et al.*, 2009).

Conventional methods of controlling codling moth can sometime become ineffective, due to an improper application of insecticides related to incorrect timing of application when the pest is not present (Postolovski & Lazarevska, 1994) or as a result of codling moth resistance to commonly used insecticides (Pajic *et al.*, 2011). In Bulgaria, Kutinkova (2010) informs that Charmillot *et al.* (2007) found resistance in diapausing CM larvae to organophosphates and pyrethroids. Also, the principles of sustainable agriculture production emphasize the concern for environment and food safety. Frequent pesticide applications create a risk of contaminating fruits with toxic residues. Started in 1991, the European Union banned the use of many insecticides and reduced the opportunity for chemical control. The use of natural biological control mechanisms could be one of the useful tools in the Integrated Pest Management (IPM) in apple orchards. Lazarevska *et al.* (2005) stresses that the number of predators and parasitoids species affecting CM is very low and represented with an extremely low number of individuals. The authors highlighted *Cleonus* sp. as the most numerous parasitoid on CM larvae in apple orchards in Republic of Macedonia. Authors concluded that poor qualitative and quantitative composition of the natural enemies is the main reason for high CM population density, therefore biological control in apple orchards does not have practical value against CM. In this situation, the pheromone based mating disruption (MD) technique could be an alternative to conventional methods of CM control. Witzgall *et al.* (2008) expressed an opinion that MD pheromones should be a tool in pest control.

Mating disruption technique is based on a female sex pheromone, which is species specific and targets only one pest species. The common mechanism of MD is a false trail following. This disrupts the male insect's sensory ability to locate the female and therefore prevent mating with females. Prevention or delay of mating causes significant impairment of breeding success with commensurate benefits in reducing subsequent crop damage. This technique has been tested since the early nineties in Switzerland and South Tyrol, Italy (Charmillot, 1990; Zingg, 2001; Veronelli & Iodice, 2004), in Slovenia (Rot & Blazic, 2005), in Fyr of Macedonia (Lazarevska *et. al.*, 2006), in Bulgaria (Kutinkova *et al.*, 2007; 2008; 2009; 2010), in Poland (Dabrowski & Olszak, 2007), in USA (Gut & Brunner, 1998) and Canada (Judd *et al.*, 1996).

The results were mostly positive when the CM population was at a low or moderate levels (Kutinkova *et al.*, 2008). In situations with high population of the pest, a combination of mating disruption and chemical treatments was recommended by Charmillot & Pasquier (2003) or the use of a combination of MD with granulosis virus product (Miñarro & Dapena, 2000; Kutinkova *et al.*, 2008; 2010).

Material and methods

The experiment was carried out during the 2014 and 2015 seasons in apple orchards in Prespa Region, south-west in Republic of Macedonia. Four apple orchards in two years were utilized for the mating disruption trials. Three of them (Experimental plot (2014, 2015), Dolna Bela Crkva (2014) and Carev Dvor (2015)) used MD technique for CM control and three of them [Evla (2014, 2015), Dolna Bela Crkva (2014) and Carev Dvor (2015)] used only chemical control and served as a reference apple orchards.

The first plot with MD techniques in 2014, named as the Experimental plot, located in village Evla was established on 1 ha in 2012 with following varieties: Red Delicious, Golden Delicious, Red Chief, Mutsu and Granny Smith. Isomate C TT dispensers (with active ingredient of (E, E)-8,10-dodecadien-1-ol) (CBC Europe, subsidiary of Shin-Etsu Japan)

were installed on this plot at a density of 500 dispensers per ha on 20 May, 2014. 20. The dispensers were hung in the upper third of the tree crown. Two insecticide treatments were applied against aphids, before the application of MD pheromones. Later in the season two more insecticide treatments were applied against green apple aphids (*Aphis pomi* de Geer) when the economic thresholds were overcome (10% of infested shoots). The first reference apple orchards in 2014 were located in Evla village on 2.8 ha, with crop varieties Idared, Red Delicious, Golden Delicious. At this reference orchard, 6 insecticide treatments were applied during the season, from the beginning of April till the beginning of September, to control CM, leafminers, leafrollers, aphids and mites. At least four to five of those treatments were timed against CM.

Second MD plot in 2014 was established in commercial apple orchard in Dolna Bela Crkva (3.4 ha). This orchard was established in 2004 with apple varieties Red Delicious, Golden Delicious, Red Chief, Mutsu and Granny Smith. Isomate C TT dispensers were installed on this plot at a density of 500 pieces per ha on 20 and 21 May, 2014. After the installation of MD dispensers, three insecticide treatments were applied during the season against green apple aphids when economic thresholds were reached. The second control apple orchards located in the same village Dolna Bela Crkva (2.8 ha), established in a period 2000-2012, with crop varieties Idared, Red Delicious, Golden Delicious, served as a reference. At the reference orchards, 9 insecticide and 2 acaricide treatments were applied during the season, from the beginning of April till the beginning of September, to control CM, leafminers, leafrollers, aphids and mites. Of those, 5 treatments were timed against CM.

In the 2015season the experiment was carried out again in Experimental plot – Evla (1 ha) and in one new commercial apple orchards in village Carev Dvor (2.8 ha). In the Experimental plot – Evla, the Isomate C TT dispensers were installed with density of 500 pieces per ha on 5 May, 2015. Four insecticide treatments were applied during the season against green apple aphids and leafrollers when economic thresholds were exceeded (10% infested shoots for aphids and 15 adult moths per pheromone trap in 15 days for leafrollers). One acaricide treatment was applied in the Experimental plot against European red mite (*Panonychus ulmi* (Koch)) when economic threshold for July was reached (6-8 mites per 100 leaves). One commercial apple orchard located in village Evla also served as a reference plot in 2015. At the reference orchards, 9 insecticide treatments were applied during the season, from the beginning of May till the first decade of September, to control CM, leafminers, leafrollers, aphids and mites with 4-5 insecticide treatments timed against codling moth.

The second MD plot in 2015 was located in the village Carev Dvor on 3.8 ha with apple varieties: Red Delicious, Golden Delicious, Red Chief, Mutsu, Granny Smith and Idared. The Isomate C TT dispensers were installed on this plot at a density of 500 pieces per ha on 5 May, 2015. Three insecticide and two acaricide applications were applied during the season against green apple aphids and European red mite when economic thresholds were reached. One commercial apple orchard in Carev Dvor, located in vicinity of trial plot with crop varieties: Red Delicious, Golden Delicious and Idared served as the reference during the 2015 season. At the reference orchard 12 insecticide treatments were applied during the season, from the beginning of May till the beginning of September, to control CM, leafminers, leafrollers, aphids and mites. At least 4-6 treatments were targeted against codling moth. In both orchards (experimental and reference) dynamics of CM flights were monitored by the use of pheromone traps. One triangular, standard trap per ha, baited with a standard capsule (Csalomone OP-72-T1-01) containing 1 mg codlemone was installed in the trial and in the reference plots prior to the beginning of flights during each season. Traps were inspected once

a week. Caught CM moths were counted and removed. The indicator for the efficiency of the MD system in experimental plots was an economic threshold of zero CM caught.

Evaluation of the MD system through the apple season was performed by counting the number of damaged fruit and was carried out once per month (July, August) in each plot on 1000 fruits before the harvest as well as during the harvest (September). Economic thresholds were established as 5 larvae per 1000 fruits in July and 8 larvae per 1000 fruits in August. The goal for the CM control during the season is to achieve fruit damage below 2%. Also, the presence of CM in dropped fruit and percent of damaged fruit were evaluated twice per season (July and September).

Elaboration of data

Data on catches of male moths in the pheromone traps were considered as total for each date of control and presented in a graphical form. The rate of fruit damage by CM was expressed as the percentage of damaged on fallen fruits during the season and as the percentage of damaged fruits at the end of the season. Collection date has been statistically evaluated by Analyze of Variance and mean separation conducted by LSD-test.

Results

The dynamics of codling moth flights during the 2014 and 2015 seasons are presented in Figures 1, 2, 3 and 4. According to the collected trap data, the duration of flights in all reference orchards in Prespa Region lasted almost four months, starting from the end of May (24^{th} to 27^{th}) during the 2014 season or the beginning of May (5^{th}) in the 2015, until the 2^{nd} or even 3^{th} week of August (14^{th} to 18^{th}) in 2014 and 5^{th} to 10^{th} August during the 2015 season with few peaks through two generations in all localities (Figures 1, 2, 3 and 4).

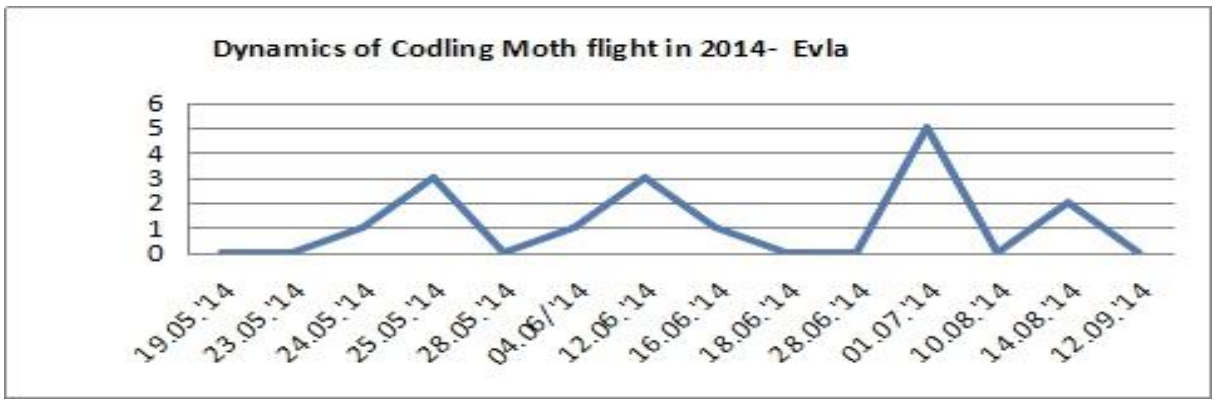

Figure 1. Dynamics of codling moth flights in the reference apple orchard Evla in 2014

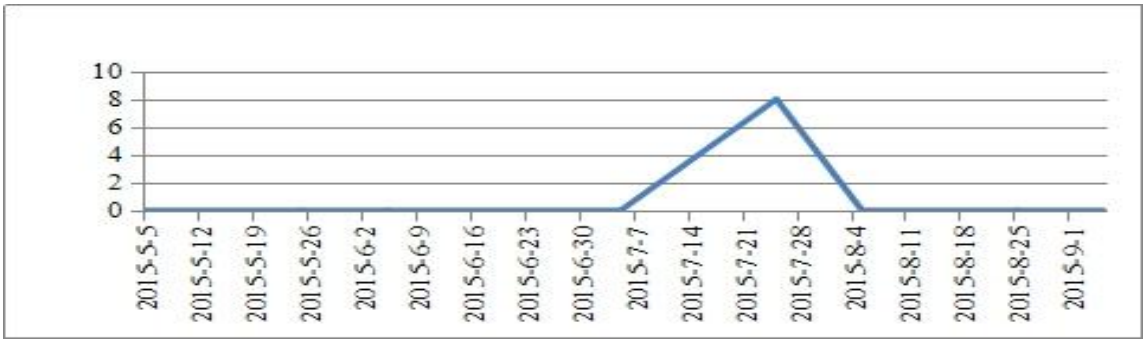

Figure 2. Dynamics of codling moth flights in the reference apple orchard Evla in 2015

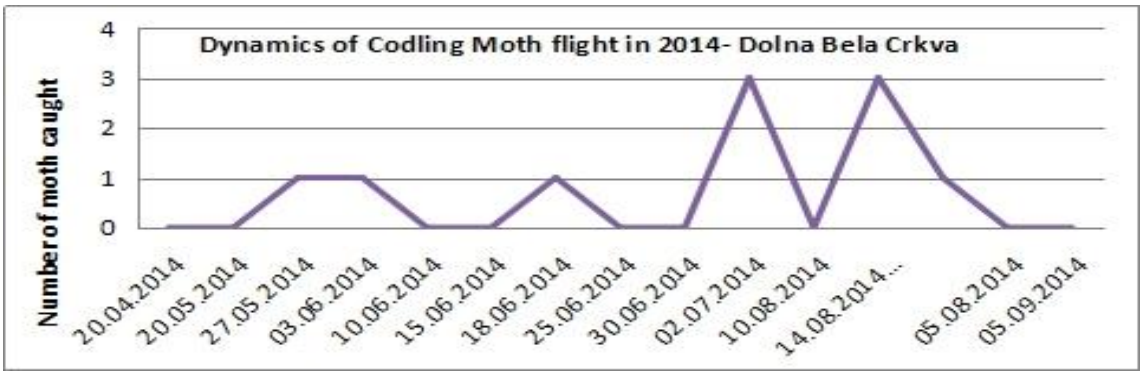

Figure 3. Dynamics of codling moth flights in the reference apple orchard Dolna Bela Crkva in 2014

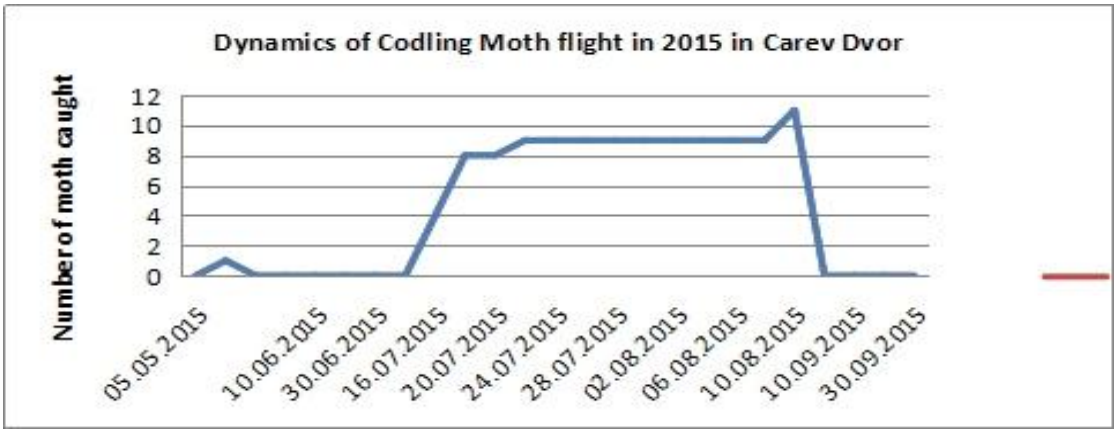

Figure 4. Dynamics of codling moth flights in the reference apple orchard in Carev Dvor in 2015

The total number of moths caught per season in the reference orchards was 16 (Evla) and 21 (Dolna Bela Crkva) during the 2014 season and 8 (Evla) and 113 (Carev Dvor) during the 2015 season. At the same time no moths were caught in the MD trial plots during both seasons.

Table 1. Percent of dropped fruit damage in 1000 fruits in Isomate CTT trials and reference apple orchards during the 2014 and 2015 seasons

2014	**Isomate CTT – Evla**		**Reference plot – Evla**		**Isomate CTT – Dolna Bela Crkva**		**Reference plot – Dolna Bela Crkva**	
	No of fallen fruits	%	No of fallen fruits	%	No of fallen fruits	%	No of fallen fruits	%
July	235	0	215	0.5	145	0	140	1.7
Sept. Pre harvest	156	0	254	0.4	120	0	115	0.8

2015	**Isomate CTT – Evla**		**Reference plot – Evla**		**Isomate CTT – Carev Dvor**		**Reference plot – Carev Dvor**	
	No of fallen fruits	%	No of fallen fruits	%	No of fallen fruits	%	No of fallen fruits	%
July	69	0	204	0.9	110	0.8	150	2
Sept. Pre harvest	57	0	167	0.6	115	0.8	107	0.9

Fruit damage by CM larvae in the reference orchard was higher than in the experimental plots, despite numerous insecticide treatments. Analysis of fallen fruits during the season has shown no damages of CM in experimental plots Evla and Dolna Bela Crkva in 2014. During the 2015 season the percentage of damaged fruits in experimental plot Evla was 0% and 0.8% in Carev Dvor (Table 1). In the reference plots the fallen fruit damage was between 0.4 and 1.7% during the 2014 season and between 0.6 and 2% during the 2015 season. The levels of damaged fruit decreased toward harvest as a result of numerous insecticidal treatments.

In the experimental plots fruit damage was not observed during the season or at the harvest during the 2014 season. The fruit damage of 0.2% was recorded in experimental plot Carev Dvor during the 2015 season. The percent of fruit damage in reference plots was considerable higher, varying from 1 to 1.6% at harvest in 2014 and 0.5 to 0.7% in 2015 (Table 2).

Table 2. Percent of fruit damage in 1000 fruits in Isomate CTT trials and reference apple orchards in 2014 and 2015 (LSD 0.05 = 0.16; LSD 0.01 = 0.234)

2014	Isomate CTT – Evla	Reference plot – Evla	Isomate CTT – Dolna Bela Crkva	Reference plot – Dolna Bela Crkva
July	0	0	0	1.5
August	0	1.2	0	1
September Pre harvest	0	1	0	1.6

2015	Isomate CTT – Evla	Reference plot – Evla	Isomate CTT – Carev Dvor	Reference plot – Carev Dvor
July	0	0.3	0	1.2
August	0	0.5	0.2	0.7
September Pre harvest	0	0.5	0.2	0.7

The number of insecticide treatments was lower in MD experimental plots and was focused only against green apple aphid and European red mite. The frequency of insecticide applications in reference plots was higher. The number of insecticide treatments in experimental plot was 3 to 7 against aphids and 1 to 2 against red mite in 2014 and 2015, respectively. The number of insecticide applications in reference plots was 6 to 7-9, or even 12 applications against aphids and CM and 2 acaricide treatments against European red mite.

The final fruit damage in experimental plots (Evla, Dolna Bela Crkva) was 0% in 2014 and 0% (Evla) to 0.2% (Carev Dvor) during the 2015 season. In reference plots the levels of fruit damage were between 1% (Evla) and 1.6% (Dolna Bela Crkva) in 2014 and 0.5% (Evla) and 0.7% (Carev Dvor) during the 2015 season (Table 2).

The obtained results proved statistical differences at 0.01 level which means that in 99 percent of cases, the conventionally treated orchards will have more fruit damaged by CM than comparable orchards protected by MD pheromones.

This conclusion is very realistic because the chemical control may have missed the proper timing for an insecticide application when the CM larvae enter the fruits, resulting in damaged fruit. Unlike the chemical control, the MD technique uses dispensers, which release the sexual pheromone continuously during the season and confuse the CM males for four months of their appearance. The possibilities of male finding female in apple orchards with MD are very low as documented with 0 percent of damaged fruit during two experimental years.

According to our results, the MD pheromones can completely control the CM population, reduce the number of insecticidal treatments and their adverse effects in environmental, and allow natural enemies such as coccinellids, chrysopids, syrphids and anthocorids to increase their populations and be successful in controlling other pest insects in apple orchards, as was confirmed with the reduced number of chemical control in experimental plots. The conventional chemical control in the reference plots successfully reduces the populations of CM, aphids and mites but the chemical pressure on the environmental was very high and with high economical cost.

Discussion

The presented results confirmed our approach to decrease the number of insecticide treatments in apple orchards and to replace the chemical control with environmentally friendly measures. Conventional chemical control in apple orchards in Prespa Region uses 6 to 12 insecticidal applications when the MD pheromone system reduces the insecticidal treatments to 3-4 applications, what reduced the negative impact on the environment. Typically, producers increase the number of insecticidal treatments as a result of their ineffective pest control or because of the emergence of pesticide resistance. Kutinkova (2010) presented the results of laboratory study by Charmillot *et al.* (2007) to point out that some of the organophosphates and pyrethroids are ineffective against CM due to the CM larvae insecticide resistance. Apple producers tend to get high profit with fruit damages below 2%, so in order to rich this goal, they increase the number of insecticidal treatments with using mostly organophosphates for the control of apple pests.

Due to the development of insecticide resistance in CM populations, the conventional control programs became less effective. At the same time, the new MD systems, as documented in the trial plots, were effective in controlling the CM population densities and in keeping the population under economic thresholds with 0-0.2% of fruit damage levels observed at the end of the season. This confirms that the pheromone-based mating disruption program provides a chance for successful reduction of CM and indirect success in reduction of the populations of other pests in apple orchards (aphids and mite) by increasing the number of natural enemies. The reduction in the number of insecticidal treatments enables natural enemies to keep the population of the aphids, leafrollers and mites under economical injury levels. Based on the results of the recent study as well as on the results obtained in other countries, this alternative MD strategy has been proposed for the control of CM in apple orchards. The incorporation of the MD technique should be the core of IPM program in apple production. This system should secure the effective control of the CM, keep the natural enemies allowing them to control other pests in apple orchards, and help avoid any pollution of the orchard environment or fruits at the same time.

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