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SUCCESSFUL APPLICATION OF THE BACULOVIRUS PRODUCT MADEX® FOR CONTROL OF *CYDIA POMONELLA* (L.) IN BULGARIA

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Abstract: The codling moth (CM), *Cydia pomonella* (L.), causes heavy damage in Bulgarian apple orchards. Conventionally treated orchards, were monitored in this study. In spite of numerous chemical treatments, these orchards showed increasing flight densities of CM moths, growing populations of hibernating larvae and rising fruit damage rates. Thus, the control of CM by conventional spraying programmes became ineffective, apparently due to the development of resistance to insecticides. Products based on the *Cydia pomonella* granulosis virus (CpGV), such as Madex®, may provide alternative control tools that can be applied with other approaches, for a sustainable control strategy. The trials were carried out in Central-South and South-East Bulgaria, in 2006-2010. Four treatments of Madex® against the first generation, and six treatments against the second generation kept the fruit damage and population density of CM at a low level. Based on the obtained results, different control strategies have been suggested, depending on the initial CM pressure in a particular orchard. Madex® may be a promising alternative to traditional programmes of CM control. Its dose, however, should be adjusted to the initial CM population density. Also, at a high or moderate CM population density Madex® applications should be combined with MD to avoid resistance of CM to granulovirus. At the peak of CM hatching, additional chemical treatments may be sometimes necessary. Such treatments include using insecticides which are still effective against CM.

Key words: apple, codling-moth, *Cydia pomonella*, mating-disruption, Isomate-C-plus, CpGV, Madex®, flight dynamics, fruit-damage

INTRODUCTION

The codling moth (CM), *Cydia pomonella* (L.), remains the principal insect pest of apple, pear and walnut in Bulgaria. Till the present time it has been controlled by routine applications of a broad spectrum of insecticides, such as organophosphates, to maintain this pest at an economically acceptable level. Disadvantages of such practices include strongly negative effects on beneficial species and eventually development of resistance to insecticides used (Sauphanor *et al*. 2000; Boivin *et al*. 2001). The presence of strong insecticide resistance was reported for codling moth strains collected from some orchards in Bulgaria (Charmillot *et al*. 2007). Increasing resistance hinders effective management of the pest and thereby threatens apple production. The intensive use of chemicals also strongly contradicts the principles of sustainable horticulture. Hence there is an obvious and urgent need for the development of less intrusive control practices, involving a non-chemical, sustainable strategy. Microbial pesticides based on the codling moth granulovirus (CpGV) (family Baculoviridae, genus *Granulovirus*) provide growers with an option that would complement mating disruption and other interventions. Such pesticides have a minimal impact on the environment and beneficial insect species (Gröner 1986; Lacey and Shapiro-Ilan 2003).

C. pomonella granulovirus virus (CpGV) is a member of the genus *Granulovirus* (family Baculoviridae) – Crook (1991). It was first discovered in CM larvae collected from orchards of apple and pear in Mexico (Tanada 1964). After ingestion of CpGV by a CM larva, the granulin dissolves in the alkaline midgut and release the virions that initiate infection in midgut epithelial cells. The virus replicates and spreads throughout the major body tissues, leading to death of the host (Federici 1997; Thiem 1997). CpGV is highly pathogenic for CM but harmless for non-target organisms. Because of these characteristics, the CpGV-based microbial agents are frequently used in biological control of CM in apple orchards in Western Europe (Baudry *et al*. 1996; Hunter-Fujita *et al*. 1998).

Results of early field trials showed that applications of CpGV lead to effects comparable to applications of chemical insecticides with regard to their ability to prevent CM damage in apples (Huber and Dickler 1977; Mantinger *et al*. 1992). The recommended rates of CpGV preparations range from 10^{11} granules per ha to $5x10^{13}$ granules per ha (Charmillot 1989; Audemard *et al*. 1992).

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CpGV is suitable for using in Integrated Pest Management (IPM) and organic 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. The CpGV product Madex®, has been registered in Bulgaria since 2005.

The objective of the present study was to test the application of CpGV in Bulgarian apple orchards and to develop suggestions for its use with a low, moderate or high population density of CM. For the testing, the efficacy of the CpGV product Madex® was assessed for control of CM during four years, in three important fruit growing regions of Bulgaria.

MATERIALS AND METHODS

The trials were carried out in three different regions of Bulgaria. (a) Kalekovets near Plovdiv, (b) Samuilovo near Sliven during the years 2006–2009 and (c) Aheloy (Burgas district) during the years 2007–2010.

The geographical location and general thermal conditions of particular sites are presented in table 1. Bulgaria is situated in the south-eastern part of the Balkan Peninsula of Europe, adjacent to the Black Sea in the East. The climate is moderately continental. So, the differences between particular locations are mainly determined by the altitude above sea level. Among the locations under study, Samuilovo has a slightly cooler climate, due to the highest altitude and Aheloy has the warmest one due to its lowest altitude.

The Madex® formula contains the granulosis virus of the codling moth in a concentration of $3x10^{13}$ granules per litre, produced by Andermatt Biocontrol AG, Grossdietwil, Switzerland. Its recommended use is either at a full dosage of 100 ml per ha ($3x10^{12}$ granules per ha) or at a half-dosage of 50 ml per ha ($1.5x10^{12}$ granules per ha).

The first experiment was set up in an isolated commercial orchard, established in 2001 near the village Kalekovets, close to the city Plovdiv in South-Central Bulgaria. This trial consisted of two parallel plots. One plot was 0.50 ha, the other one was 1.4 ha. The plots were separated by rows of cherry trees between them. Apple cultivars grown there were: Goldspur, Braeburn, Revena, Golden Delicious, Golden Rider, Melrose, Pinova and Florina. According to the grower, codling moth pressure before the beginning of the trials (in 2005) was relatively low – under the economical threshold, *i.e.* below 1.5–2% of damaged fruits at harvest. In the trial, the Madex® treatments were combined with fungicide treatments till the end of June, and then from July, the Madex® treatments were applied alone, without the fungicides. Applications were carried out at 10–17-day intervals – five against the first generation and five against the second CM generation in 2006 and 2007, and four against the first, and six against the second generation in 2008, always using a half-dosage of Madex®, *i.e.* 50 ml per ha.

The second experiment was carried out in a commercial apple orchard, established in 1988 in the town Aheloy, the Burgas district of South-East Bulgaria, on a 0.1-ha plot. Apple cultivars grown there were: Melrose and Mutsu. According to the growers, codling moth pressure was relatively high in the previous year (from 5 to 30%) in the conventionally treated rows. However the population density was not estimated by means of corrugated paper band traps. In the part of this orchard (1.0 ha) where Madex® had been applied since 2007 (trial plot), the population density of CM was 3.7 larvae per tree and the percentage of damaged fruits before the harvest 2006 was 5.3%. The trial was carried out in the time period from 2007–2010. Madex® was applied, starting from the second week of May and then at 11–14-day intervals. Altogether there were 10 applications – 4 against the first generation and 6 against the second generation, at a full-dosage of 100 ml/ha.

The third experiment was set up in a commercial apple orchard, established in 1990 in the village Samuilovo, the Sliven district of South-East Bulgaria, on a 0.8-ha plot. Granny Smith, Macspur and Melrose cultivars were grown in this orchard. According to the manager of the orchard, codling moth pressure was high; above 30% of the fruits were damaged at the harvest of 2005. The trials at this site were carried out in the 2006–2009 time period. The trials involved a combination of Madex® spray applications with mating disruption by installation of Isomate C plus dispensers of the Japanese company Shin-Etsu. The dispensers were installed on the trial plot at a density of 1,000 pieces per ha: on April 24 in 2007, on April 19 in 2008 and on April 23 in 2009. They were hung in the upper third of the tree canopy. Madex® was applied at a dosage of 100 ml per ha ($3x10^{12}$ granules per ha), 10 times in each season, at 10-14-day intervals – starting from the 2nd week of May.

As a reference for every trial, a typical commercial orchard was selected in the same region. From 14 to 18 conventional insecticide treatments, mostly organophospates and pyrethroids were applied every season, in these typical commercial orchards. Most of the treatments were aimed against the codling moth.

For monitoring of CM flights, in each trial and respective reference plot, two triangular traps were installed each spring, before the CM flights started. The traps were baited with a standard capsule (Pheronet OP-72-T1-01) containing 1 mg of codlemone. They were inspected twice a week, the caught moths were counted and removed.

Table 1. Location and mean yearly temperature at the experimental sites

Trial No.	Location of trial plot	Region of Bulgaria	Altitude north	Altitude [m a.s.l.]	Mean yearly temperature
1	Kalekovets	Plovdiv distr., South-Central	42°23′	~ 150	12.3°C
2	Aheloy	Burgas distr., South-East	42°65′	~ 50	15.0°C
3	Samuilovo	Sliven distr., South-East	42°58′	~ 190	12.4°C

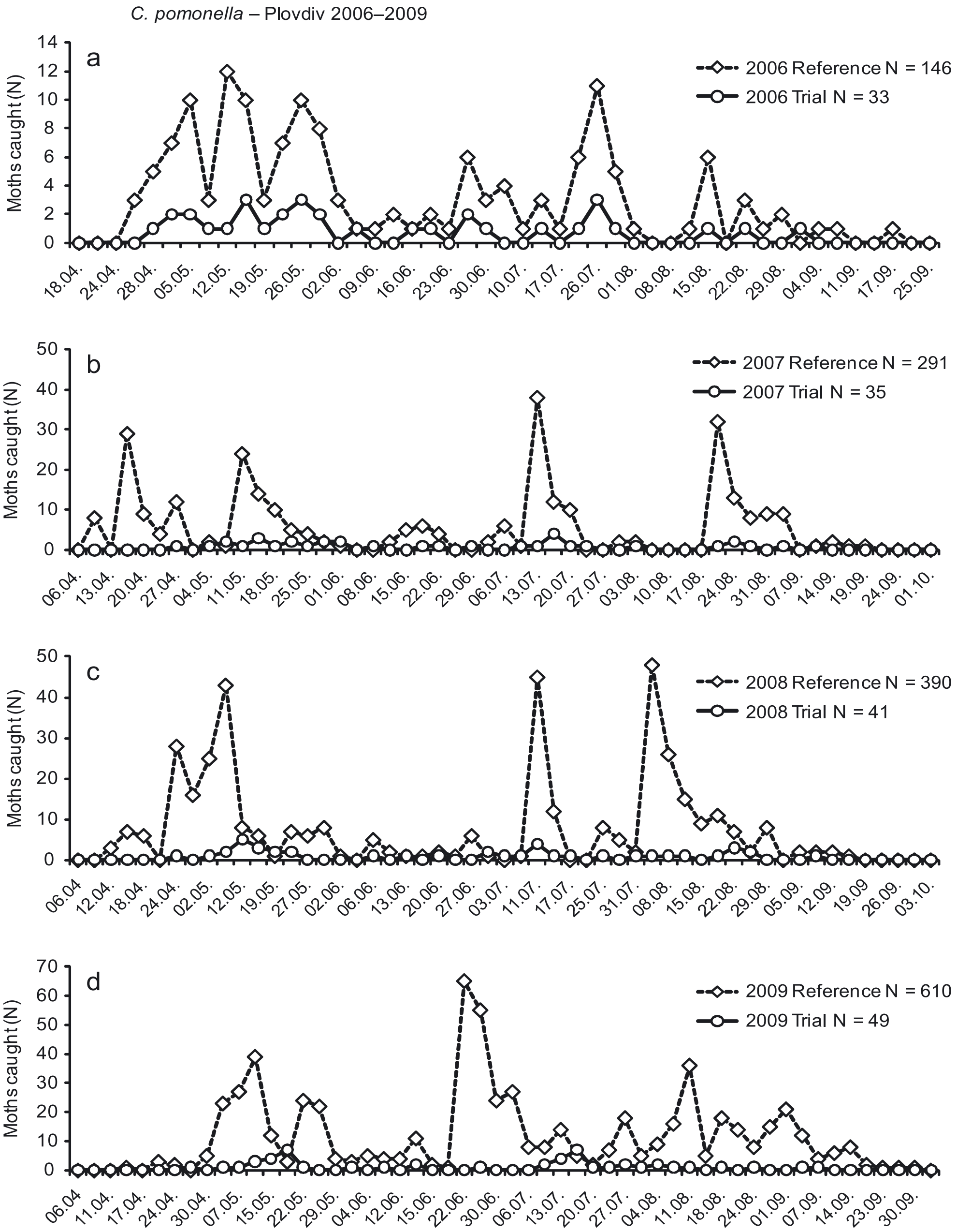
a.s.l. – meter above sea level

Every June, corrugated cardboard band traps were wrapped around the trunks of 30 or 40 representative trees in each trial and reference orchard. They were recovered in autumn after harvest, in order to count the hibernating populations of CM.

Fruit damage by CM larvae was evaluated periodically during the season, starting from the beginning of June and finishing before harvest on 1,000 or 2,000 apples on the trees. At harvest, the final fruit damage was estimated on samples of 2,000 or 3,000 apples randomly taken from the crop of every trial and orchard plot. The percentages of damaged apples were calculated and subjected to statistical analysis, using the chi-square test.

RESULTS AND DISCUSSION

On the site located near Plovdiv (Central-South Bulgaria) the codling moth flights started in 2006 in the 3rd week of April (Fig. 1a–d, Table 2). In 2007 and 2008, CM flights started in the 2nd week of April. In 2009, the flights started in the 4th week of April. The last catches of male codling moths were recorded in pheromone traps in the 3rd week of September in 2006–2008 and at the end of September in 2009. At the site near Plovdiv, the codling moth developed two full and probably a partial third generation. The latter was indicated by the incidence of slight, but pronounced peaks of the catches of CM at the end of August and a steady continuation of flights in Sep-



N – number of moths caught

Fig. 1. Dynamic of *C. pomonella* flights in the reference orchard and in the Madex treated plotin Central-South Bulgaria (near Plovdiv)

Table 2. Dates of first and last flights in the conventionally treated, orchards in different regions, in 2006–2010

Year	Kalekovets, Plovdiv district		Aheloy, Burgas district		Samuilovo, Sliven district	
	first flight	last flight	first flight	last flight	first flight	last flight
2006	Apr 26	Sept 17	May 3	Sept 4	May 2	Sept 9
2007	Apr 10	Sept 19	Apr 27	Sept 18	Apr 30	Sept 14
2008	Apr 12	Sept 15	Apr 25	Sept 15	Apr 25	Sept 14
2009	Apr 15	Sept 30	Apr 29	Sept 29	Apr 30	Sept 25
2010	–	–	Apr 28	Oct 8	–	–

Table 3. Total catches of *C. pomonella* moths in two pheromone traps in thereference and trial plots, at different sites, in consecutive years

Year	Kalekovets, Plovdiv district		Aheloy, Burgas district		Samuilovo, Sliven district	
	trial	reference	trial	reference	trial	reference
2006	33	146	–	78	0	128
2007	35	291	95	252	0	283
2008	41	390	128	307	0	357
2009	49	433	130	342	0	630
2010	–	–	133	492	–	–

Table 4. Number of hibernating larvae per tree in the trial and reference plots, at different sites, in different years of study

Year	Kalekovets, Plovdiv district		Aheloy, Burgas district		Samuilovo, Sliven district	
	trial	reference	trial	reference	trial	reference
2006	0.20	3.32	3.70	4.90	d.m.	2.16
2007	0.28	7.97	0.25	6.43	1.05	4.58
2008	0.53	9.82	0.85	8.58	1.65	9.03
2009	0.50	11.63	0.65	9.34	1.28	12.27
2010	–	–	0.50	11.16	–	–

d.m. – data missing in this year

Table 5. Evolution of fruit damage in the trial and plot at Kalekovets and in the reference orchard near Plovdiv, 2006–2009

2006				2007				2008				2009			
Date	trial	refe-rence	chi-square	date	trial	refe-rence	chi-square	date	trial	refe-rence	chi-square	date	trial	refe-rence	chi-square
June 1	0.00	0.2	0.499	June 2	0.00	0.20	0.499	June 5	0.00	0.2	0.499	June 4	0.00	0.5	3.19
p = 0.480				p = 0.480				p = 0.480				p = 0.074			
June 19	0.00	0.8	6.09	June 23	0.00	21.3	192.3	June 25	0.00	1.6	13.9	June 23	0.00	2.1	18.8
p = 0.014				p < 0.001				p < 0.001				p < 0.001			
July 31	0.00	2.3	20.8	July 5	0.00	0.0	–	July 5	0.00	0.9	7.07	July17	0.00	2.7	24.7
p < 0.001				–				p = 0.008				p < 0.001			
Aug 8	0.00	2.5	22.7	July 27	0.03	2.70	17.4	July 29	0.3	2.1	11.9	Aug 9	0.4	5.6	42.1
p < 0.001				p < 0.001				p < 0.001				p < 0.001			
Aug 22	0.06	3.3	17.0	Aug 10	0.08	4.70	25.5	Aug 11	0.4	4.7	33.7	Aug 24	0.6	8.6	64.9
p < 0.001				p < 0.001				p < 0.001				p < 0.001			
Sept 28	0.04	5.9	44.8	Aug 31	0.1	11.2	101.5	Aug 29	0.6	12.8	102.6	Sept 6	0.8	14.5	112.6
p < 0.001				p < 0.001				p < 0.001				p < 0.001			
Oct 6	0.06	6.8	48.5	Sept 27	0.3	18.7	161.9	Sept 27	0.7	33.8	274.4	Oct 4	0.9	38.4	304.6
p < 0.001				p < 0.001				p < 0.001				p < 0.001			
pre-harvest	0.04	5.9	44.8	pre-harvest	0.1	11.2	101.5	pre-harvest	0.6	12.8	102.6	pre-harvest	0.8	14.5	112.6
	p < 0.001				p < 0.001				p < 0.001				p < 0.001		
at harvest	0.06	6.8	68.5	at harvest	0.3	18.7	161.9	at harvest	0.7	33.8	274.4	at harvest	0.9	38.4	304.6
	p < 0.001				p < 0.001				p < 0.001				p < 0.001		

tember every year. This trend was observed already in our earlier studies on the same site (Kutinkova *et al*. 2009).

In general, codling moth appeared with high intensities in the conventionally treated reference orchard in Central-South Bulgaria. The total number of moths caught in pheromone traps was high and successively increased throughout the years of the study (Table 3).

At the same time only incidental moths were caught in the trial plot established at Kalekovets, where Madex® was applied in 10 sprays at 50 ml per ha. The total number of moths caught during the season on the trial plot was low (Fig. 1a–d, Table 3). These results were the first indication that the trial was successful. The low catch was apparently due to a relatively low CM population density in the trial plot. The obtained results are in accordance with the reports of Audemard *et al*. (1992) and Baudry *et al*. (1996), who noted that a low initial CM population density was important for a high efficacy of CpGV treatment. Pasquier and Charmillot (1998) also pointed out that at a low initial population density (< 1 CM larva per tree) it is possible to maintain the pest population at a low level for a long time, by means of CpGV.

The overwintering population of CM at the Kalekovets trial (Madex) plot remained at a low level during the years of the study, with only a slight increase in 2008 and 2009 (Table 4). It should be stressed that the population density of CM in the trial plot at this location, was at a low level when the experiment with CpGV started, and remained low (<1 larva per tree) till the end of the trial. At the same time, in the reference orchard the CM population rapidly increased – from 3.32 larvae per tree in 2006 up to 11.63 larvae per tree in 2009.

Fruits damaged by CM larvae appeared in the reference plot near Plovdiv starting from the beginning of June. The percentage of damage successively increased; at harvest it reached the values disqualiying the crop (Table 5). It is worth noting that the fruit damage in the reference steadily increased during the years of the study. In the trial plot only few damaged fruits were found during the season. The final fruit damage (at harvest) in the trial plot never exceeded the economical threshold in force in Bulgaria (below 1.5–2%). Each year of the study, the damage rates differed between the treated and the reference orchards already after the first few assessments in June. The damage rates stayed significantly different until harvest (Table 5, Chi-square tests).

When the initial population density of codling moth was relatively low (< 1 larvae per tree) and the percentage of damaged fruits was below the economical threshold in the preceding years, it was possible to control CM at Kalekovets with Madex®. This refers to Madex® applied even at the lower recommended dose.

In the second trial, located at Aheloy (Burgas district, South-East Bulgaria), flights of CM in the reference varied depending on weather conditions. In general, CM flights in this region started 7–17 days later than in Central-South Bulgaria. At Aheloy, the first moths appeared at the end of April or at the beginning of May. Flights continued with numerous peaks, through two generations, till the 4th week of September or even till the beginning of October (Fig. 2a–d, Table 6). Small flight peaks, occurring at the end of August every year – most markedly in 2009, might indicate the incidence of a partial third generation also in this region. Even before starting the trial, the overwintering population at Aheloy, reached 3.7 larvae per tree. In the first year of application, Madex® reduced the CM population in the trial plot to 0.25 larvae per tree; then it fluctuated, never exceeded¸however, the threshold value of 1 larva per tree (Table 4). At the same time, in the conventionally treated part of the orchard, which served as a reference for this trial, the number of larvae collected from corrugated paper bands was very high and steadily increased from 2007 to 2010.

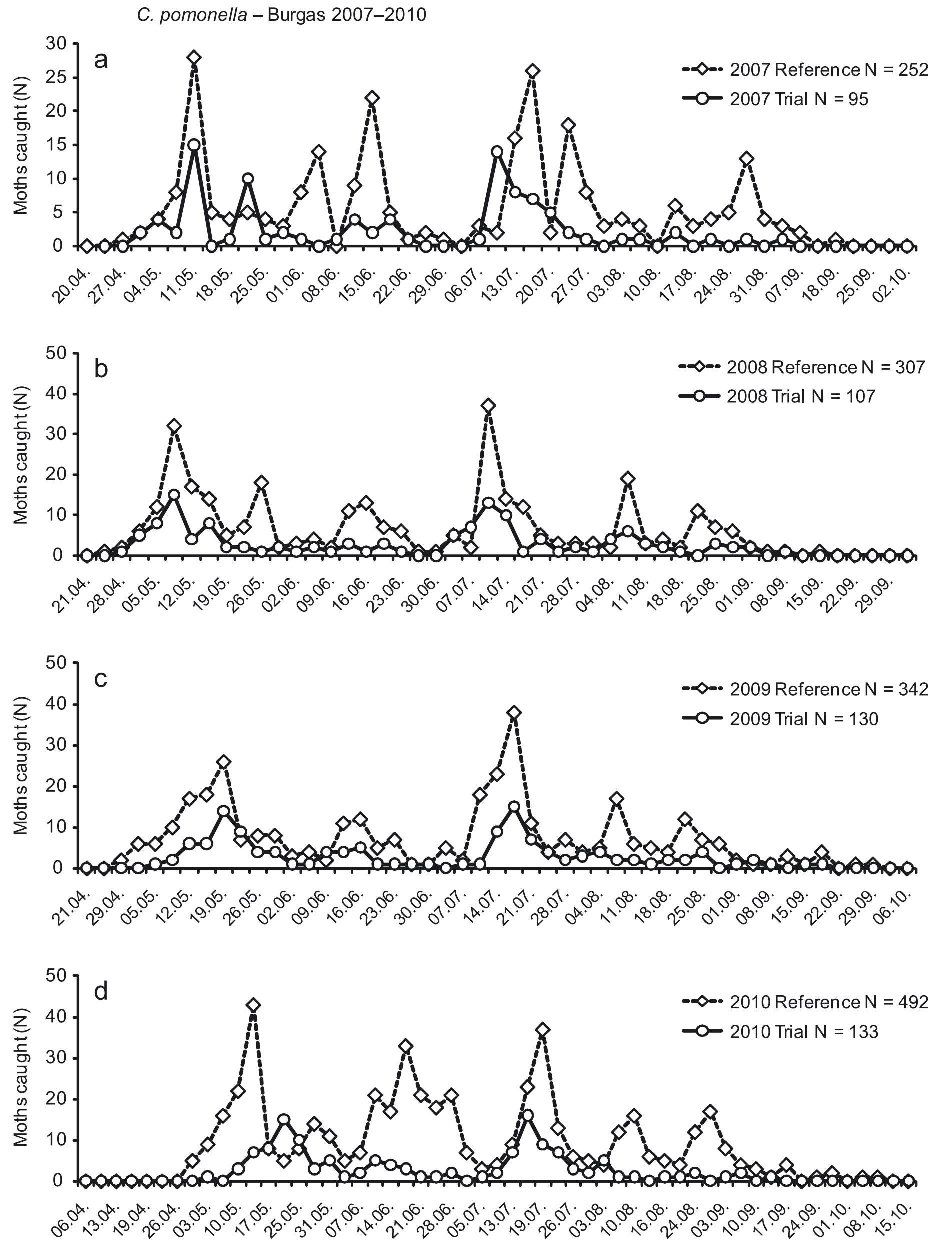
CM larvae infested only few fruits in the trial plot during the season, starting from the end of June (Table 6). The final fruit damage (at harvest) never exceeded the economical threshold value (below 1.5–2%). At the same time fruit damage in the reference plot was high and markedly increased from 2008 to 2010. In all the years, the damage rates differed between treated and reference orchards after the first few assessments of damage in June and stayed significantly different thereafter until harvest (Table 6, Chi-square tests).

In the region of the third trial, located in Samuilovo near Sliven, flights of CM in the reference orchard near Sliven varied depending on the season. In this region, flights lasted from the end of April or the beginning of May till the 2nd– 4th week of September, through two full generations (Fig. 3, Table 2). As at the other experimental sites, small peaks of flights were observed at the end of August every year. Such peaks may indicate the occurrences of a partial third generation in this region, too. In the reference plot, the total number of caught moths was high and rapidly increased throughout the years of the study (Table 3). At the same time, in the trial plot, treated with Madex® and MD, no male moths were caught during the season in any year. These results confirm the efficacy of the mating disruption by pheromone dispensers.

The hibernating CM larvae population at the Samuilovo trial plot was not recorded in the first year. Later (in 2007–2009) the hibernating population it was recorded at a moderate level (Table 4). At the same time in the reference orchard near Sliven a high number of overwintering larvae was noted. The numbers increased from year to year, reaching above 12 larvae per tree in the autumn 2009.

Fruit damage on the reference plot followed the trend of the hibernating larvae population. Numerous fruits damaged by CM larvae were recorded in successive years earlier and earlier. At harvest, CM larvae also damaged a higher percentage of the crop every year (Table 7). In the trial plot, treated simultaneously with MD and Madex®, the fruit damage was rather low, only slightly exceeding 2% at harvest. Again, in each year of the study, the damage rates differed between treated and reference orchards already at the first assessments in June and stayed significantly different thereafter until harvest (Table 7, Chi-square tests).

Monitoring of CM flights, carried out in three different regions confirm our earlier findings (Kutinkova *et al*. 2009, 2010), indicating that codling moth in Bulgaria has not only two full generations, but also a partial third generation.



N – number of moths caught

Fig. 2. Dynamics of *C. pomonella* flights in the reference orchard and in the Madex treated plotin South-East Bulgaria (Burgas district)

A clear tendency to a rapid increase of CM pressure in the reference orchards was noted in all trials, as proved by the increase in the catches of male moths, hibernating larvae and fruit damage rate. This is in line with the data presented in our earlier publications (Kutinkova *et al*. 2009, 2010). The findings indicate that programmes of CM control based on conventional insecticides have become ineffective. Overall development and spread of resistant CM strains appears to be the reason for the ineffectiveness of the conventional insecticides. Resistance of the larvae, collected in some Bulgarian orchards, to organophosphates and pyrethroids has been confirmed in laboratory tests by Charmillot *et al*. (2007). Now, it is clear that the phenomenon of resistance is overwhelming.

The presented results have shown that the virus product Madex® may effectively control codling moth when its population density is relatively low. This is in accordance with the reports of Pasquier and Charmillot (1998), Stara and Kocourek (2003) and Tamm *et al*. (2004). So, the products based on the CM granulosis virus (CpGV), such as Madex®, may be used as an alternative to conventional insecticides. However, development of resistance to CpGV presents a threat that must also be considered. Recently, single orchards with CM populations showing about a 1,000-fold decrease in susceptibility of CM to CpGV were noted in Switzerland, Germany and France (Charmillot and Pasquier 2003; Fritsch *et al*. 2005; Sauphanor *et al*. 2006). Since resistance can also develop against new versions of Madex, a consequent anti-resistance strategy for use of virus products has been suggested by Zingg (2008). Available control measures against codling moth should be combined according to the situation in a particular orchard. The Integrated Pest Management (IPM) farmers should combine pheromone mating disruption with a virus treatment. In the case of high population densities some chemical control measures

Table 6. Evolution of fruit damage in the trial and reference plot at Aheloy, Burgas region, South-East Bulgaria, 2007–2010

2007				2008				2009				2010			
Date	trial	refe-rence	chi-square	date	trial	refe-rence	chi-square	date	trial	refe-rence	chi-square	date	trial	refe-rence	chi-square
June 9	0.00	0.5	3.19	June 8	0.00	0.30	1.33	June 11	0.00	0.60	4.15	June 7	0.00	0.50	3.19
p = 0.074				p = 0.249				p = 0.042				p = 0.074			
June 29	0.05	1.5	4.00	June 22	0.10	1.30	8.57	June 25	0.20	1.60	9.29	June 23	0.20	1.40	7.49
p = 0.045				p = 0.003				p = 0.002				p = 0.006			
July 15	0.00	2.1	18.8	July 19	0.20	2.50	17.7	July 14	0.40	2.80	16.3	July 16	0.60	2.30	8.69
p < 0.001				p < 0.001				p < 0.001				p = 0.003			
Aug 1	0.60	2.2	7.91	Aug 5	0.30	3.20	22.0	Aug 9	0.50	4.50	29.7	Aug 8	0.80	4.60	24.66
p = 0.005				p < 0.001				p < 0.001				p < 0.001			
Aug 16	0.80	6.5	41.4	Aug 18	0.80	7.20	47.7	Aug 21	0.70	8.50	61.6	Aug 23	1.40	9.40	54.8
p < 0.001				p < 0.001				p < 0.001				p < 0.001			
Sept 6	0.45	18.3	294.5	Sept 8	0.90	21.3	168.3	Sept 7	1.20	22.7	172.6	Sept 5	1.60	21.5	153.2
p < 0.001				p < 0.001				p < 0.001				p < 0.001			
Oct 2	0.50	20.4	325.0	Oct 5	1.30	27.5	209.3	Oct 3	1.70	31.6	232.2	Oct 4	1.90	31.7	228.2
p < 0.001				p < 0.001				p < 0.001				p < 0.001			
pre-	0.45	18.3	294.5	pre-	0.90	21.3	168.3	pre-	1.20	22.7	172.6	pre-	1.60	21.5	153.2
harvest	p < 0.001			harvest	p < 0.001			harvest	p < 0.001			harvest	p < 0.001		
at	0.50	20.4	325.0	at	1.30	27.5	209.3	at	1.70	31.6	232.2	at	1.90	31.7	228.2
harvest	p < 0.001			harvest	p < 0.001			harvest	p < 0.001			harvest	p < 0.001		

Table 7. Evolution of fruit damage in the trial plot at Samuilovo and in its reference orchard near Sliven, 2006–2009

2006				2007				2008				2009			
Date	trial	refe-rence	chi-square	date	trial	refe-rence	chi-square	date	trial	refe-rence	chi-square	date	trial	refe-rence	chi-square
June 1	0.0	0.5	3.19	June 9	1.2	0.8	0.44	June 8	0.2	0.6	1.1	June 10	0.3	0.5	0.124
p = 0.074				p = 0.51				p = 0.29				p = 0.725			
June 15	0.0	1.5	13.0	June 21	3.8	3.5	0.051	June 21	1.3	3.7	10.3	June 23	1	3.8	14.8
p < 0.001				p = 0.82				p = 0.001				p < 0.001			
July 13	0.0	1.8	15.9	July 5	0	0	–	July 18	0.9	2.6	7.17	July 15	0.2	2.3	15.8
p < 0.001								p = 0.007				p < 0.001			
Aug 4	0.0	2.4	21.8	July 29	0.8	5.8	35.2	Aug 10	1.6	10.5	60.4	Aug 09	1.1	9.6	62.6
p < 0.001				p < 0.001				p < 0.001				p < 0.001			
Aug 23	0.05	4.1	26.0	Aug 12	1	9.8	66.5	Aug 23	1.8	14.5	90.2	Aug 26	1.4	15.7	109.0
p < 0.001				p < 0.001				p < 0.001				p < 0.001			
Sept 14	0.35	5.2	38.3	Sept 05	2.1	13.2	73.6	Sept 07	2	23.8	163.0	Sept 04	1.6	21.5	153.2
p < 0.001				p < 0.001				p < 0.001				p < 0.001			
Oct 6	0.50	6.1	44.3	Oct 3	2.5	14.8	82.5	Oct 3	2.2	28.4	207.4	Oct 5	2	31.7	453.6
p < 0.001				p < 0.001				p < 0.001				p < 0.001			
pre-	0.35	5.2	38.3	pre-	2.1	13.2	73.6	pre-	2	23.8	163.0	pre-	1.6	21.5	153.2
harvest	p < 0.001			harvest	p < 0.001			harvest	p < 0.001			harvest	p < 0.001		
at	0.50	6.1	44.3	at	2.5	14.8	82.5	at	2.2	28.4	207.4	at	2	31.7	453.6
harvest	p < 0.001			harvest	p < 0.001			harvest	p < 0.001			harvest	p < 0.001		

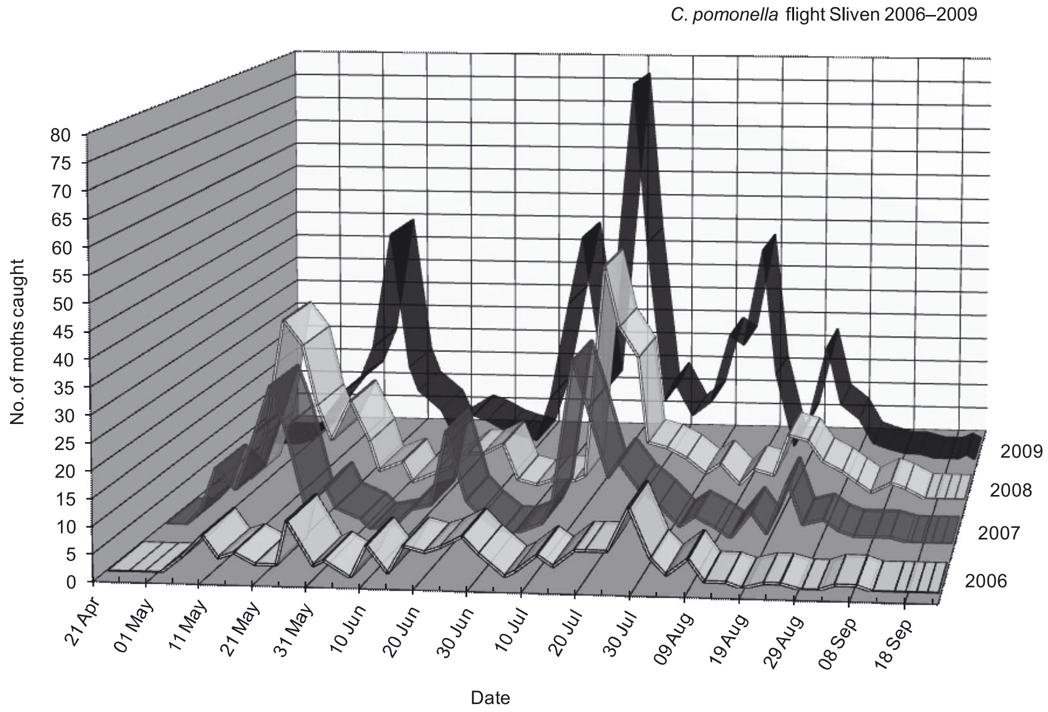


Fig. 3. Dynamics of *C. pomonella* flights in the reference orchard for the trials in Samuilovo near Sliven

should also be included. The organic farmers have less of a choice. Therefore, it is especially important that codling moth populations are kept at a low density to maintain optimal conditions for the use of mating disruption along with the virus applications.

It is suggested that at a low population density (< 1 larva per tree, < 1% of damaged fruits in the preceding year) Madex® may be applied at a dose of 50 ml/ha against the first and second generation. Altogether, that means 10 treatments per season. The use of the lower recommended dose may minimise the risk of the development of resistance (Zingg 2008). For an optimal effect, combination of virus treatment with mating disruption (MD) should be applied. Then, number of Madex treatments may be reduced to 3–5 per generation. MD shows its full efficacy at low population levels (Gut and Brunner 1998). At a moderate population density of CM (1–3 larvae per tree, 1–5% of damaged fruits), as an effective and safe measure, it is suggested using mating disruption by pheromone dispensers in combination with 4–6 treatments of Madex® at the full dose rate (100 ml/ha). Additional chemical treatments can be applied at the peak of CM egg hatching to reduce the damage level more quickly.

At a high population density of CM (> 3 larvae per tree, > 5% of damaged fruits) – mating disruption + Madex® at the full rate (100 ml/ha) and obligatorily additional treatments with insecticides, which are still effective, should be applied. These can be insect growth regulators acting on eggs (*e.g.* fenoxycarb) or larvae (moulting inhibitors or ecdyson mimics), but also products which are newly available on the market.

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