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New insights into the biology and ecology of *Cydia pomonella* from apple orchards in Croatia

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Abstract

The biology and ecology of three codling moth, *Cydia pomonella* L. (CM), populations from Croatia were investigated. The effects of current and past climate regimes on the behaviour of the three CM populations were investigated, and the changes in their biology and ecology were observed. The first population investigated was free from chemical control treatment, whereas the other two populations were subjected to chemical control. Climate data were obtained from LUFT climatological stations in Croatia. A later emergence of adult moths in the spring, later oviposition and later emergence of larvae occurred in the untreated orchard versus the treated orchards. In the untreated orchard, two generations of CMs were observed per year, whereas an additional third flight period of the moths was observed in the treated orchards. The results of this study confirm the possibility that a third generation of the pest can develop in Croatia in years in which the sum of degree-day is higher than average.

Key words: *Cydia pomonella*, codling moth, Tortricidae, apple orchard.

Introduction

Codling moth (CM) (*Cydia pomonella* L.) is a key pest in apple fruit production. This pest attacks pears, walnuts, quince and certain stone fruits in addition to apples, causing economic losses in fruit production (Ciglar, 1998).

The pest was originally present in Eurasia; however, during the past two centuries, it has dispersed globally with the commercial cultivation of apples and pears and has become one of the most successful pest insects known (Franck *et al*., 2007; Thaler *et al*., 2008). The codling moth has adapted successfully to different habitats by developing various ecotypes and populations, which are often designated by the term *strain* and which differ in several morphological, developmental and physiological features (Meraner *et al*., 2008).

Although this pest has been present in Croatia since ancient times (Kovačević, 1952), little is known about its biology and ecology in the region. In Croatia, two generations of CMs appear per year (Kovačević, 1952; Ciglar, 1998; Maceljski, 1999). However, during certain growing seasons in Northern Croatia, first-generation individuals enter diapause as mature fifth-instar larvae and do not have a second generation (Maceljski, 1999).

The moth overwinters as a fully grown larva within a thick, silken cocoon that can be found under loose scales of bark and in the soil or debris around the tree base (Alford, 1984). The larvae pupate inside their cocoons in early spring when the temperatures exceed 10 °C. Depending on the temperature, pupal development occurs within 7-30 days. For the development of adults, 100 degree-days measured from the 1st of January are required (Wildbolz, 1962); this value is usually attained at the end of April. For the development of a generation from the egg stage until the appearance of the moths, 610 degree-days are required (Wildbolz, 1962). The second generation appears after ten days and its flight lasts from mid-July to mid-August. The moths lay eggs again during this period, repeating the developmental cycle.

Recently, Croatian apple orchards have sustained considerable damage due to CM populations, and an increase in the population growth of this pest has been observed (Barić and Pajač, 2009). CM monitoring using pheromone traps has in the past decade revealed earlier times of moth flight. This was found to be correlated with the higher ambient daily temperatures which were recorded in this period (Barić *et al*., 2008). During 1999, CM males in Northwest Croatia were captured until the end of July; recently, however, male flight has been observed until the end of September (Barić and Pajač, 2009). This increase in the distribution and abundance of CMs in Croatia is similar to what was observed in other parts of Europe and elsewere around the world (Barnes, 1991; Polesny, 2000; Charmillot and Pasquier, 2003)

Based on the literature it is hypothesised that global warming and chemical-resistant CM biotypes are responsible for the longer flight period and the observed increase in the abundance of CMs. Moreover, it is possible that this pest is developing an additional (third) generation in Croatia.

The abundance of CMs cannot be explained by any single ecological factor (Geier, 1963). Climate changes resulting in higher temperatures and a higher total annual temperature may affect the biology of this pest. The long-term use of insecticides with similar mechanisms of action may produce genetic changes (frequencies of genes and genotypes) within the populations of this pest.

The aim of this study was to analyse the biology and ecology of CM from apple (*Malus domestica* Borkh.) orchards by: 1) studying its development during three growing seasons; 2) recording the climatic data during the past decade in Northwestern Croatia; and 3) demonstrating the possible development of a third generation.

Materials and methods

The study was conducted during the 2008, 2009 and 2010 growing seasons in two commercial orchards and in one extensive production orchard with different agro-ecological production conditions.

The orchards are situated in the largest apple-producing region of Croatia, near the borders of Hungary and Slovenia (figure 1).

The orchards in this region range from 70 to 250 ha in area, and the apples are produced according to the principles of integrated pest management (IPM) (Pajač *et al*., 2011). The three sampled orchards were differentiated based on their insecticide treatments. Orchard 1 (Beloslavec) was characterised by a low cultivation level without any insecticide applications and the apple production in this orchard was classified as untreated orchard. This orchard is surrounded by natural pasture without any organised agricultural production. Orchards 2 (Kloštar Ivanić) and 3 (Nedelišće) were characterised by intensive cultivation in accordance with IPM procedures and sprayed with organophosphates, insect growth regulator (IGR) insecticides and neonicotinoid insecticides seven times during the growing season (treated orchards). Since the studied orchards differ in size, a central 1 ha area was chosen for trap placement in each orchard to minimise variation in the experiment.

The Kloštar Ivanić orchard was established 15 years ago and the Nedelišće orchard has the longest integrated control programme running of all three orchards investigated. The geographic distance between the orchards are as follows: Beloslavec to Kloštar Ivanić is 55 km; Beloslavec to Nedelišće is 60 km; and Kloštar Ivanić to Nedelišće is 110 km. Hence, the CM populations are assumed to be isolated from one another. In all three orchards Idared, Golden Delicious and Jonagold apple varieties were grown.

CM biology

The development of CM during the growing seasons of 2008, 2009 and 2010

One hundred plant organs (leaves and fruits) were examined for the presence of eggs every week throughout the growing period. The presence of larvae was observed by inspecting 1000 fruits of the Idared variety for damage according to EPPO (EPPO Standards, 2004) standards. Trees were randomly selected for examination at the time of ripening and all of the fruits were harvested. Overwintering populations were monitored by collecting diapausing larvae from 20 cardboard traps placed on the main trunk of each tree in the middle and on the edges of the orchards.

CM flight dynamics

The appearance of CM males was observed using delta sticky pheromone traps (Csalomon®). The pheromone traps were placed in the middle and on the edges of the chosen areas in the orchards. A total of three traps were placed in each orchard. According to the manufacturer's instructions, the attractant range of the traps is approximately 500 m; thus, it was assumed that the sampled moths were from the population that existed in those orchards only.

The traps were placed in the three orchards during the second half of April, before the appearance of the first flight of CM was theoretically possible. The pheromone traps were changed every four weeks. The catch of moths from all three traps was counted every two days. Monitoring of adult CM was conducted until the beginning of October.

For each season, the captures were compared with captures from 1997 to 1999 (3 years) in the Kloštar Ivanić orchard and captures from 1993, 1997, 1998 and 1999 (4 years) in the Nedelišće orchard. Past data only exist for the commercial orchards, i.e. Kloštar Ivanić and Nedelišće, and thus, the extensive production orchard, Beloslavec, was not included.

CM ecology

The comparison of the Phenology Model Database (Wildbolz, 1962) with the observational field data

Climatic data on the mean average daily temperatures were collected from LUFT climatological stations (data not shown). This data was used to calculate the expected emergence of each life stage of CM using the Wildbolz theoretical model (1962).

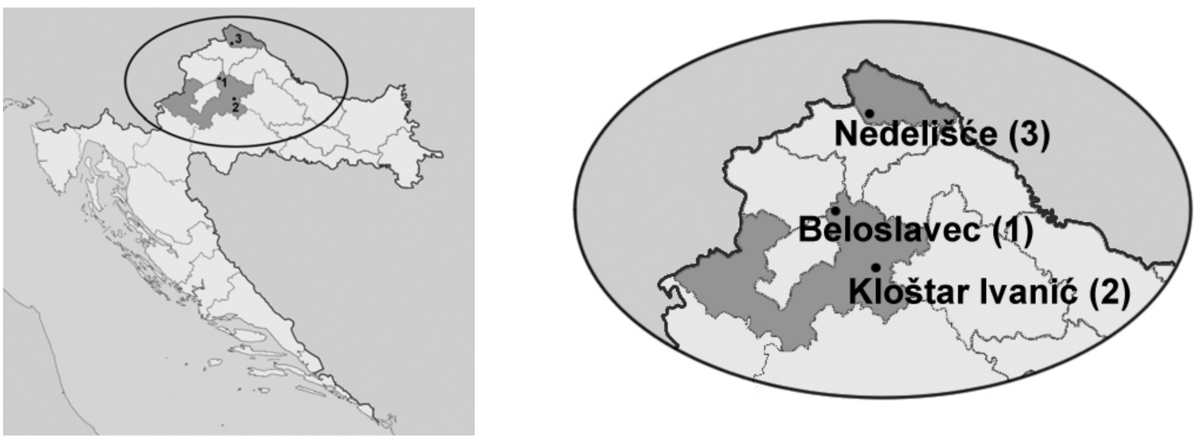

Figure 1. The location of the three study sites/orchards (and populations) from which *C. pomonella* individuals were collected. 1: Beloslavec (untreated); 2: Kloštar Ivanić (treated); 3: Nedelišće (treated).

The model is based on calculating the sum of daily mean air temperatures reduced by 10 °C (the thermal threshold for development of CM).

According to Wildbolz (1962) the thermal constant, on average 100 degree-days measured from the 1st of January is required for the development of adults. Emergence of larvae from eggs is expected at 90 degree-days reported from the appearance of the first eggs observed. For the development of a generation from the egg stage until the appearance of the moths an average of 610 degree-days are required.

Calculated predictions were compared with actual appearances of CM life stages observed in the field.

Results

CM biology

The development of CM during the growing seasons of 2008, 2009 and 2010

The data of the present three-year study on the developmental stages of CMs in three apple orchards (Beloslavec, Kloštar Ivanić and Nedelišće) are shown in tables 1, 2 and 3.

The emergence of the moths was later in the spring in the untreated orchard (Beloslavec) (table 1) than in the two treated orchards (Kloštar Ivanić and Nedelišće) (tables 2 and 3). Later oviposition observed by visual leaf inspection was also in accordance with the later emergence of the moths, and on average, the larvae also appeared later in the untreated orchard (Beloslavec). The late appearance of the larvae resulted in the later emergence of the second generation of moths. The start of the second flight was monitored through the development of larvae in cardboard traps and adult catches on pheromone traps (table 1).

In the untreated orchard, an additional third flight of CMs was not noted over the three-years. We assume that the larvae of the second generation went into diapause after the completion of their development.

In the Kloštar Ivanić orchard, an additional or potential third flight period of CM was observed during all three of the growing seasons (table 2). An additional flight of CM was also recorded in 2009 in the Nedelišće orchard (table 3). In the Kloštar Ivanić and Nedelišće orchards the additional flight of moths was determined by monitoring the development of larvae in cardboard traps and adult catches on pheromone traps. Oviposition and the development of larvae were determined by the visual inspection of fruit (tables 2 and 3).

CM flight dynamics

On average, CM flight started later from 1993 to 1999 than from 2008 to 2010 in treated orchards (i.e. Kloštar Ivanić and Nedelišće). In the years 2008, 2009 and 2010, CM flight started earlier and ended later in the season. Different situation has been observed in untreated orchard (i.e. Beloslavec) where flight distribution data from 2008 to 2010 strongly resembles the flight distribution data from 1993 to 1999 in treated orchards (i.e. Kloštar Ivanić and Nedelišće) (tables 4, 5 and 6).

From 1993 to 1999, CM flight began in early May (tables 5 and 6), whereas it began two to four weeks earlier during 2008, 2009 and 2010, in mid-April (tables 5 and 6).

From 1993 to 1999 CM flight ended in mid-August (tables 5 and 6), whereas flight was prolonged in recent years (i.e. 2008, 2009 and 2010) and lasted until the beginning of September (tables 5 and 6).

Table 1. Comparison of Phenology Model Database and field monitoring of *C. pomonella* in the untreated orchard Beloslavec.

		2008			2009			2010		
Pest stage		Emergence observed (date)	Degree-day accumulations (temp. in °C > 10)	Emergence expected (Wildbolz, 1962)	Emergence observed (date)	Degree-day accumulations (temp. in °C > 10)	Emergence expected (Wildbolz, 1962)	Emergence observed (date)	Degree-day accumulations (temp. in °C > 10)	Emergence expected (Wildbolz, 1962)
I. Generation	Adults	07.05.	104.60	06.05.	27.04.	148.50	17.04.	30.04.	114.20	29.04.
	Eggs	09.05.	113.70		01.05.	162.80		03.05.	136.50	
	Larva	23.05.	201.90	21.05.	14.05.	252.30	07.05.	23.05.	221.00	14.05.
II. Generation	Adults	13.07.	774.90	09.07.	03.07.	713.20	04.07.	08.07.	694.40	10.07.
	Eggs	15.07.	790.70		05.07.	737.50		10.07.	719.60	
	Larva	25.07.	877.80	16.07.	15.07.	841.50	14.07.	16.07.	816.70	16.07.
Potential III. Generation	Adults	not noticed		03.09.	not noticed		22.08.	not noticed		07.09.
	Eggs	not noticed			not noticed			not noticed		
	Larva	not noticed		11.09.	not noticed		31.08.	not noticed		24.09.
Total sum of degree-days (1st January - 30th September)			1,454.50			1,704.90			1,439.40	

Table 2. Comparison of Phenology Model Database and field monitoring of *C. pomonella* in the treated orchard Kloštar Ivanić.

Pest stage		2008 Emergence observed (date)	2008 Degree-day accumulations (temp. in °C > 10)	2008 Emergence expected (Wildbolz, 1962)	2009 Emergence observed (date)	2009 Degree-day accumulations (temp. in °C > 10)	2009 Emergence expected (Wildbolz, 1962)	2010 Emergence observed (date)	2010 Degree-day accumulations (temp. in °C > 10)	2010 Emergence expected (Wildbolz, 1962)
I. Generation	Adults	16.04.	46.50	03.05.	14.04.	85.30	17.04.	23.04.	67.90	28.04.
	Eggs	19.04.	56.20		16.04.	97.20		26.04.	87.30	
	Larva	07.05.	116.10	18.05.	07.05.	183.70	08.05.	10.05.	178.50	12.05.
II. Generation	Adults	10.06.	409.20	05.07.	15.06.	543.10	03.07.	23.06.	533.00	09.07.
	Eggs	12.06.	427.10		17.06.	566.70		26.06.	555.60	
	Larva	22.06.	520.80	12.07.	28.06.	658.30	13.07.	04.07.	658.00	15.07.
Potential III. Generation	Adults	28.07.	951.40	23.08.	27.07.	999.60	22.08.	11.08.	1.111,10	02.09.
	Eggs	29.07.	963.80		29.07.	1024.90		15.08.	1.162,40	
	Larva	04.08.	1.049,10	02.09.	05.08.	1.121,40	30.08.	23.08.	1.252,10	18.09.
Total sum of degree-days (1st January - 30th September)			1,490.50			1,677.10			1,457.40	

Table 3. Comparison of Phenology Model Database and field monitoring of *C. pomonella* in the treated orchard Nedelišće.

Pest stage		2008 Emergence observed (date)	2008 Degree-day accumulations (temp. in °C > 10)	2008 Emergence expected (Wildbolz, 1962)	2009 Emergence observed (date)	2009 Degree-day accumulations (temp. in °C > 10)	2009 Emergence expected (Wildbolz, 1962)	2010 Emergence observed (date)	2010 Degree-day accumulations (temp. in °C > 10)	2010 Emergence expected (Wildbolz, 1962)
I. Generation	Adults	21.04.	36.80	13.05.	14.04.	56.50	27.04.	30.04.	77.50	04.05.
	Eggs	28.04.	49.70		16.04.	65.70		02.05.	93.60	
	Larva	17.05.	138.00	26.05.	09.05.	156.80	14.05.	23.05.	180.40	24.05.
II. Generation	Adults	21.07.	794.30	22.07.	09.06.	397.90	21.07.	12.07.	674.90	21.07.
	Eggs	23.07.	804.50		11.06.	416.40		14.07.	704.70	
	Larva	30.07.	884.10	31.07.	20.06.	506.90	29.07.	20.07.	787.90	31.07.
Potential III. Generation	Adults	not noticed		31.08.	11.08.	1.049,70	22.09.	not noticed		
	Eggs	not noticed			12.08.	1.061,00		not noticed		
	Larva	not noticed		08.09.	20.08.	1.154,20	09.10.	not noticed		
Total sum of degree-days (1st January - 30th September)			1,349.60			1,461.30			1,304.10	

CM ecology

The comparison of the Phenology Model Database (Wildbolz, 1962) with the observed field data

Compared with the results from the Phenology Model Database (Wildbolz, 1962), our field CM monitoring in the Kloštar Ivanić orchard showed an earlier emergence of diapausing generations of moths in the spring than was theoretically expected. The moths appeared at 46.5 (2008), 85.3 (2009) and 67.9 degree-days (2010).

In all three years of study, the emergence of the third generation of pests was observed from both the field data and the sum of temperatures above the threshold (from 1st of January to 30th of September) (table 2). For example, the Nedelišće orchard showed an earlier emergence of CMs in the spring (from 36.8 to 77.5 degree-days) compared with the theoretical Phenology Model Database (Wildbolz, 1962). The monitoring data on the appearance of the larvae agreed with the expected 90 degree-days in all three years of study (table 3).

The flight capture data (table 6) shows the possible development of a third pest generation during the 2009 growing season. In 2009, theoretical considerations correctly predicted the emergence of a third generation (from the 1st of January to the 30th of September, 1461.3 degree-days were collected) (table 3). In contrast, for the untreated orchard Beloslavec, the monitoring data for the flight captures revealed a situation that was completely different from the observations at the treated sites (table 4). Although the degree-days value was favourable for the development of a third generation of CM, an additional generation did not appear (table 1).

Table 4. *C. pomonella* adults caught during the growing seasons 2008, 2009 and 2010 in the Beloslavec orchard.

2008		2009		2010	
Date	Captures	Date	Captures	Date	Captures
07.05.	1	27.04.	1	30.04.	1
09.05.	2	29.04.	3	02.05.	0
12.05.	2	01.05.	0	04.05.	2
14.05.	3	03.05.	2	06.05.	2
16.05.	3	05.05.	0	08.05.	3
18.05.	6	07.05.	3	12.05.	0
20.05.	8	09.05.	4	14.05.	1
22.05.	7	12.05.	0	16.05.	1
24.05.	8	14.05.	4	18.05.	4
26.05.	12	16.05.	5	20.05.	3
28.05.	9	18.05.	3	22.05.	0
30.05.	6	20.05.	5	24.05.	5
04.06.	4	24.05.	0	26.05.	7
06.06.	5	28.05.	7	28.05.	8
08.06.	8	30.05.	5	01.06.	5
10.06.	6	02.06.	8	04.06.	4
12.06.	5	04.06.	7	06.06.	6
14.06.	3	08.06.	9	08.06.	3
16.06.	4	10.06.	7	12.06.	5
18.06.	3	12.06.	12	16.06.	2
20.06.	5	14.06.	12	18.06.	4
22.06.	4	16.06.	10	22.06.	0
24.06.	4	20.06.	7	24.06.	3
26.06.	2	22.06.	5	28.06.	2
30.06.	3	24.06.	3	30.06.	1
05.07.	1	26.06.	1	02.07.	0
08.07.	0	30.06.	0	04.07.	0
10.07.	0	02.07.	0	06.07.	0
13.07.	8	03.07.	4	08.07.	5
15.07.	7	06.07.	4	10.07.	3
19.07.	6	08.07.	3	12.07.	0
21.07.	3	10.07.	5	16.07.	6
23.07.	5	14.07.	5	18.07.	9
26.07.	4	18.07.	6	20.07.	5
29.07.	6	20.07.	7	24.07.	8
01.08.	5	22.07.	5	28.07.	7
03.08.	5	25.07.	4	30.07.	8
05.08.	4	29.07.	2	04.08.	10
09.08.	4	01.08.	3	06.08.	7
12.08.	3	04.08.	4	10.08.	5
14.08.	2	06.08.	3	12.08.	4
20.08.	3	08.08.	2	16.08.	6
23.08.	2	11.08.	1	18.08.	3
				20.08.	0
				24.08.	2
				26.08.	1
				30.08.	0
				02.09.	0
				04.09.	1

In the treated orchards, the CM moths appeared in the spring before they were theoretically expected, whereas the appearance of the moths at the Beloslavec orchard occurred slightly later than anticipated (tables 1 and 4).

Discussion and conclusions

The codling moth biology and ecology in Northwestern Croatia changed between 1993 and 2010. As seen in the comparison of the flight dynamics of CMs during the past decade (from 1993 to 1999) to the present (2008, 2009 and 2010), the moths from the overwintering generation were observed much earlier in the spring during the past three years. Moreover, the temporal duration of the flight of the moths increased. Worner (1991) reported that studies using time-series analysis and multivariate techniques to analyse the influence of climate on insect populations continue to confirm that climatic factors have a significant influence on how population fluctuations. Changes in the population dynamics of insects are caused by a number of factors, but at least in temperate climates, the temperature is considered to be the most important factor affecting the developmental rate, fecundity and mortality of insects (Worner, 1992).

Shel'deshova (1967) reported that the codling moth was of significant importance as a pest in regions where the sum of the degree-days exceeded 750 (threshold temperature 10 °C) (i.e. where complete development of the population is assured). The sum of seasonal temperature values above 10 °C in Croatia found during the period of this study ranged from 1,304.10 to 1,704.90 degree-days (tables 1 and 3), indicating very favourable conditions for the development of this pest in this region.

Rafoss and Sæthre (2003) confirmed that an increase in temperature could cause a major change in the abundance and pest status of CM.

During our three-year study, we did not observe additional flights of the hypothesised third generation in the untreated orchard despite the similar climatic conditions found among the three orchards. Therefore, we can conclude that the insecticide treatments may be responsible for the changes in the behaviour of this pest in the treated orchards.

Due to climate change and frequent insecticide treatments, CM populations are assumed to have differentiated into many ecotypes with different biological and physiological requirements for their development (Thaler *et al*., 2008).

As a consequence of frequent insecticide treatments, CMs developed resistance to several chemical groups of insecticides (Sauphanor *et al*., 2000; Boivin *et al*., 2001; Bouvier *et al*., 2001; Brun-Barale *et al*., 2005). Cross-resistance between chitin inhibitors, moult-activating compounds, such as tebufenozide, and juvenile hormone analogues were detected in the CM populations of Southeastern France (Sauphanor and Bouvier, 1995; Sauphanor *et al*., 2000). Resistance to different classes of insecticides has also been documented in CM populations from Italy (Ioriatti *et al*., 2000; 2005).

Table 5. *C. pomonella* adults caught during the growing seasons 1997, 1998, 1999, 2008, 2009 and 2010 in the Kloštar Ivanić orchard.

1997		1998		1999		2008		2009		2010	
Date	Captures	Date	Captures	Date	Captures	Date	Captures	Date	Captures	Date	Captures
12.05.	30	28.04.	47	06.05.	28	16.04.	1	14.04.	2	23.04.	8
14.05.	19	30.04.	2	10.05.	8	18.04.	1	16.04.	10	28.04.	13
16.05.	10	04.05.	76	12.05.	7	21.04.	6	20.04.	23	30.04.	19
17.05.	7	06.05.	21	14.05.	13	23.04.	1	23.04.	30	03.05.	21
19.05.	26	08.05.	23	20.05.	6	25.04.	7	25.04.	30	06.05.	17
21.05.	15	12.05.	4	22.05.	6	28.04.	9	29.04.	28	10.05.	19
23.05.	17	14.05.	42	24.05.	3	30.04.	29	04.05.	26	12.05.	11
26.05.	17	15.05.	6	31.05.	1	02.05.	8	06.05.	34	14.05.	1
28.05.	2	18.05.	20	01.06.	5	05.05.	7	08.05.	33	21.05.	10
05.06.	1	20.05.	2	08.06.	5	09.05.	33	11.05.	26	24.05.	16
07.06.	3	22.05.	34	28.06.	1	12.05.	11	13.05.	1	28.05.	2
09.06.	7	25.05.	7	02.07.	6	14.05.	8	18.05.	17	01.06.	9
11.06.	8	28.05.	14	05.07.	5	16.05.	4	21.05.	10	08.06.	6
12.06.	9	01.06.	1	07.07.	2	19.05.	9	25.05.	33	11.06.	3
16.06.	6	03.06.	3	21.07.	3	23.05.	3	27.05.	3	14.06.	5
17.06.	1	05.06.	4			26.05.	4	29.05.	2	16.06.	22
19.06.	1	08.06.	8			28.05.	18	03.06.	4	18.06.	3
23.06.	5	15.06.	1			30.05.	12	09.06.	1	23.06.	34
25.06.	7	19.06.	2			03.06.	2	15.06.	4	28.06.	1
26.06.	1	23.06.	5			10.06.	9	17.06.	2	29.06.	6
30.06.	5	25.06.	4			11.06.	3	19.06.	2	03.07.	2
04.07.	1	30.06.	16			13.06.	8	26.06.	1	05.07.	23
10.07.	1	03.07.	1			16.06.	2	29.06.	2	07.07.	22
11.07.	1	07.07.	23			20.06.	1	01.07.	10	10.07.	2
14.07.	5	10.07.	4			23.06.	3	04.07.	7	12.07.	8
15.07.	7	14.07.	5			26.06.	6	06.07.	21	15.07.	5
17.07.	3	17.07.	6			04.07.	2	09.07.	24	17.07.	1
18.07.	3	20.07.	11			08.07.	5	13.07.	23	20.07.	13
21.07.	2	21.07.	4			11.07.	14	15.07.	19	22.07.	7
23.07.	5	28.07.	24			14.07.	16	17.07.	3	27.07.	12
25.07.	7	30.07.	24			17.07.	6	27.07.	16	29.07.	3
28.07.	6	31.07.	15			24.07.	1	31.07.	8	03.08.	4
31.07.	2	03.08.	22			28.07.	8	03.08.	5	08.08.	2
01.08.	1	04.08.	7			30.07.	4	06.08.	3	11.08.	10
04.08.	8	10.08.	4			01.08.	11	10.08.	7	13.08.	4
06.08.	6	12.08.	4			04.08.	13	12.08.	7	16.08.	6
08.08.	9	14.08.	3			06.08.	6	19.08.	1	20.08.	3
11.08.	10	17.08.	19			11.08.	3	21.08.	6	23.08.	2
12.08.	2	19.08.	3			13.08.	8	24.08.	1	25.08.	2
16.08.	1	21.08.	3			18.08.	6	28.08.	1	27.08.	1
18.08.	7					20.08.	4	29.08.	2	30.08.	1
21.08.	3					22.08.	1				
25.08.	1					25.08.	2				
26.08.	1					27.08.	2				
27.08.	1					01.09.	3				
28.08.	1					08.09.	6				

Studies of the resistance of CM to chemical insecticides were not conducted on the moths investigated in this study, but it is reasonable to hypothesise that such changes could have occurred in Croatia. The insecticide treatments that have been most often applied did not achieve satisfactory results despite their correct application (Pajač *et al*., 2011). Resistance mechanisms are multiple and their individual or cumulative effects in a single population are not completely understood (Reyes *et al*., 2007).

Intra-population variability in the seasonal regulation of insect life cycles has been shown to result partly from genetic changes (Boivin *et al*., 2003). Selection for insecticide resistance in CM populations resulted from allelic substitutions at two to three loci in the Southeastern French populations of this species (Boivin *et al*., 2003). However, such an adaptive process has been associated with increased heterogeneity in the develop-

Table 6. *C. pomonella* adults caught during the growing seasons 1993, 1997, 1998, 1999, 2008, 2009 and 2010 in the Nedelišće orchard.

1993		1997		1998		1999		2008		2009		2010	
Date	Captures	Date	Captures	Date	Captures	Date	Captures	Date	Captures	Date	Captures	Date	Captures
10.05.	1	06.05.	1	08.05.	1	04.05.	1	21.04.	1	14.04.	2	30.04.	6
12.05.	1	08.05.	1	10.05.	1	06.05.	1	29.04.	1	15.04.	1	03.05.	3
14.05.	1	12.05.	2	12.05.	3	08.05.	1	05.05.	2	16.04.	2	13.05.	5
18.05.	4	14.05.	2	14.05.	1	16.05.	1	07.05.	1	18.04.	1	25.05.	5
20.05.	6	16.05.	2	16.05.	1	22.05.	1	12.05.	3	22.04.	3	29.05.	3
22.05.	6	18.05.	7	22.05.	1	24.05.	2	14.05.	2	25.04.	1	01.06.	1
24.05.	3	20.05.	2	24.05.	2	26.05.	2	15.05.	1	04.05.	4	09.06.	1
26.05.	3	22.05.	2	26.05.	1	28.05.	1	16.05.	1	05.05.	2	11.06.	1
02.06.	1	24.05.	3	30.05.	1	30.05.	4	19.05.	5	06.05.	1	14.06.	2
04.06.	1	26.05.	1	04.06.	1	04.06.	1	23.05.	1	07.05.	1	18.06.	4
06.06.	1	04.06.	2	08.06.	1	10.06.	3	27.05.	2	08.05.	3	01.07.	1
08.06.	2	08.06.	1	10.06.	2	12.06.	1	29.05.	1	11.05.	10	12.07.	2
10.06.	4	10.06.	1	16.06.	1	04.07.	1	30.05.	1	12.05.	11	15.07.	5
12.06.	7	12.06.	4	22.06.	1	06.07.	1	02.06.	4	14.05.	7	26.07.	3
14.06.	1	16.06.	2	24.06.	1	12.07.	1	05.06.	1	15.05.	7	29.07.	2
16.06.	2	18.06.	2	26.06.	1	26.07.	3	09.06.	1	18.05.	9	03.08.	1
18.06.	2	20.06.	2	28.06.	2	28.07.	1	10.06.	2	19.05.	10	09.08.	9
20.06.	1	26.06.	2	02.07.	1	02.08.	1	11.06.	2	20.05.	6	23.08.	1
22.06.	2	30.06.	6	14.07.	4	04.08.	3	12.06.	4	25.05.	4		
02.07.	1	02.07.	3	16.07.	1	06.08.	8	13.06.	2	09.06.	1		
04.07.	1	04.07.	11	24.07.	2	08.08.	5	20.06.	3	10.06.	4		
06.07.	6	08.07.	8	26.07.	5	10.08.	4	23.06.	7	12.06.	2		
08.07.	1	10.07.	2	28.07.	2	18.08.	3	24.06.	1	15.06.	2		
10.07.	1	12.07.	1	08.08.	1			26.06.	8	16.06.	2		
12.07.	2	16.07.	2					27.06.	3	19.06.	3		
14.07.	1	18.07.	5					28.06.	3	23.06.	8		
16.07.	1	24.07.	3					30.06.	2	26.06.	3		
18.07.	2	26.07.	1					02.07.	1	29.06.	2		
20.07.	1	28.07.	3					03.07.	1	02.07.	3		
22.07.	23	04.08.	2					04.07.	1	04.07.	2		
24.07.	23	06.08.	4					09.07.	2	06.07.	1		
26.07.	2	08.08.	4					21.07.	8	08.07.	1		
28.07.	3	10.08.	2					23.07.	12	11.07.	3		
30.07.	3	12.08.	1					29.07.	1	13.07.	3		
02.08.	7	16.08.	2					01.08.	3	14.07.	3		
04.08.	1	18.08.	1					07.08.	4	17.07.	15		
06.08.	3							08.08.	4	21.07.	1		
08.08.	1							11.08.	2	24.07.	7		
								12.08.	1	11.08.	1		
								13.08.	1	12.08.	1		
								14.08.	2	22.08.	2		
								18.08.	3	03.09.	1		
								22.08.	1				

mental responses to climatic factors, such as temperature (Boivin *et al*., 2003).

In previous studies, microsatellite markers were used to investigate genetic structure and gene flow among populations of moths from the same orchards (Beloslavec, Kloštar Ivanić and Nedelišće) in Croatia (Pajač *et al*., 2011). The CM populations from the three Croatian locations revealed low estimates of genetic structure despite differences in the type of control/management (treated vs. untreated apple orchards), indicating high levels of gene flow and movement. Genetic differentiation across the studied geographical regions was low (F_{ST} values ranging from 0.02 to 0.04) (Pajač *et al*., 2011). This result is consistent with the findings of previous studies of CM populations in Europe and elsewhere (Buès *et al*., 1995; Franck *et al*., 2007; Fuentes-Contreras *et al*., 2008; Franck and Timm, 2010). The findings cited here confirm the general hypothesis of a lack of genetic differentiation across populations of lepidopteran pests.

Although the variability among the orchards was relatively low, the genetic variation was significantly partitioned within the individuals in all of the categories examined (the management type and the second and third generations of each population), indicating high allelic diversity. Although the differences in allelic richness were not statistically significant, the CM population from the untreated orchard had the greatest average allelic diversity compared with the treated orchards, and certain alleles appeared only in the untreated orchard (Pajač *et al*., 2011). This finding indicates that insecticide treatments potentially lower allelic richness.

To date, not all of the possible mechanisms that can influence the changes in the behaviour of this pest are known, and the genes responsible for chemical resistance have not been characterised.

Evidently, it is possible that a third generation of the pest can develop in Croatia in years in which the sum of the degree-day is higher than average and at locations that are subjected to intensive chemical control treatments. Nevertheless, genetic analyses suggest that the moths from treated sites undergo changes in their genetic structure that positively enhance their reproductive ability and generally influence their biology.

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