Phenology, ethology and distribution of Pseudococcus comstocki, an invasive pest in northeastern Italy

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Abstract

Pseudococcus comstocki (Kuwana) is a polyphagous mealybug native to Asia. It is a pest of ornamentals and fruit trees (apple, pear and peach) in America and eastern Europe where this species has been incidentally introduced. It was first recorded in western Europe (Italy and France) in 2004. In summer 2006, additional foci of this mealybug were discovered in northeastern Italy on ornamental plants and in peach orchards. Its phenology and distribution have been investigated in northeastern Italy. *P. comstocki* develops three generations per year and overwinters in the egg stage. Overwintered eggs hatch from April onward and crawlers infest leaves and apple and pear flowers. The adult females of the three generations were observed in June (1st generation), late July-August (2nd generation), and from late September to November (3rd generation). Most of adult females move from leaves to old branches and the trunk to lay eggs. In infested orchards females are often concealed in the fruit calyx (on pears and apples) or at the fruit stem cavity (on peaches) and after fruit picking are transported far away by fruit trading. Indirect damage, i.e. honeydew excretion and development of sooty mould is remarkable, leading to early defoliation and fruit quality deterioration. Monitoring carried out on mulberry trees and ornamentals over an area of 150 km² in the Veneto Region demonstrates that these plants can act as permanent foci of this species in the territory.

Key words: Pseudococcidae, Comstock mealybug, phenology, peach orchards, ornamentals.

Introduction

The Comstock mealybug (CMB) *Pseudococcus comstocki* (Kuwana) (Rhynchota Pseudococcidae) is a highly polyphagous species native to eastern Asia and widely distributed in that region. It was incidentally introduced in central Asia and eastern Europe (Russia, Moldova, Ukraine, Georgia), in the USA, Canada, Brazil and Argentina (Meyerdirk and Newell, 1979; Romanchenko and Belskaya, 1981; Ben-Dov *et al.*, 2011). It is a notorious pest of *Morus* spp., *Catalpa* spp., fruit trees and ornamentals in the countries where it was incidentally introduced.

In the USA, CMB was first recorded in 1918 in California and New York on ornamentals (*Catalpa* spp., *Morus* spp.). In the subsequent years CMB spread in the eastern USA and in Canada (Ontario); it caused severe damage to ornamentals (mainly on *Catalpa*, *Lonicera*, *Acer*, *Aesculus*, *Ilex*, *Taxus*) and became a recognized pest of fruit trees (e.g., apple, pear, peach, apricot) (Hough, 1925; Woodside, 1936; Cox, 1940; Phillips, 1961; Agnello *et al.*, 1992; Kosztarab, 1996).

CMB was first recorded in western Europe in 2004, respectively in northeastern Italy (Verona, Veneto Region) and southern France (Bouches du Rhone and Hérault) (Pellizzari, 2005; Kreiter and Germain 2005). In both cases the mealybug was collected in urban areas off mulberry trees (*Morus nigra* and *Morus kagaya-mae*). In 2006, additional CMB outbreaks were discovered in northeastern Italy (Treviso town and several locations close to the Garda Lake) on ornamentals, i.e. *Hypericum* sp., *Prunus laurocerasus*, and *Viburnum tinus*. Infestations occurred in commercial peach orchards located near Verona (Pellizzari *et al.*, 2008; Visigalli *et al.*, 2008) and in pear and apple orchards in

Emilia-Romagna Region (Masi *et al.*, 2010; Reggiani *et al.*, 2011). Infested apple orchards were recorded also in southern France (Delgado, 2008; Groussier-Bout *et al.*, 2011), whereas in Croatia (Dalmatia) CMB was firstly reported off *Pittosporum* in 1965 and recently intercepted on pear fruits imported from China (Masten Milek and Simala, 2008; Masten Milek *et al.*, 2009).

CMB overwinters in the egg stage and develops 2-4 generations per year, depending on meteorological conditions. In Ukraine (Odessa Region) it has two generations per year and infests mainly catalpa and mulberry, rarely fruit trees (Romanchenko and Belskaya, 1981). In New York State, where CMB was a serious pest of pears (Agnello et al., 1992), it has two generations per year and the adult females are present respectively in late June-mid July (1st generation) and late August-mid September (2nd generation). In Virginia, where it is a pest of apples CMB has three generations per year (Hough, 1925; Kosztarab, 1996). In California (USA) where it is mainly found on mulberry (but also reported with high infestations on lemon, pomegranate and quince) three to four generations per year are completed (Meyerdirk and Newell, 1979a; 1979b). In China (Henan Province) CMB has 3 generations per year and apple is one of its main hosts (Liu, 2004).

CMB is a threat in commercial orchards of northeastern Italy where 30% of Italian temperate fruit production is concentrated. This suggested the necessity to study its phenology and distribution patterns around the first detected infested area and within an infested peach orchard, in order to assess adequate monitoring methods and planning Integrated Pest Management. At the same time a survey of CMB parasitoids was carried out in the infested areas in 2007 and 2008. This survey highlighted the presence of a parasitoids complex including

Year	Insecticides	Date of treatment		
000015	1 1 . 51 1	(day/month)		
2003 M1	neral oil + Fluvalinate	18/3		
	Acephate	18/4, 22/4		
	Imidacloprid	6/5		
	Azinfos-methyl	8/5, 19/5		
	Methomyl	6/6		
	Carbaryl	3/7, 10/7, 19/7, 28/7, 4/8		
2004 Mi	neral oil + Fluvalinate	17/3		
	Methomyl	27/4, 18/6, 31/7		
	Azinfos-methyl	25/5, 18/6, 13/7, 22/7		
	Etofenprox	5/6		
	Deltamethrin	15/7, 30/7, 14/8		
	Carbaryl	6/8		
2005 Mi	neral oil + Fluvalinate	23/3		
	Methomyl	22/4, 17/6		
	Azinfos-methyl	6/5, 17/6, 14/7, 22/7		
	Imidacloprid	26/5		
	Lufenuron	18/5		
	Carbaryl	11/6, 22/7, 5/8		
	Triflumuron	2/7, 9/7		
	Deltamethrin	11/8		
2006Mi	neral oil + Fluvalinate	25/3		
	Methomyl	20/4		
	Chlorpyriphos	3/5, 25/7, 4/8		
	Imidacloprid	12/5		
	Azinfos-methyl	19/7		
	Deltamethrin	25/7, 4/8, 14/8		
2007Mi	neral oil + Fluvalinate	5/3		
	Methomyl	28/3, 10/5, 30/5, 12/7		
	Thiacloprid	14/4		
	Azinfos-methyl	30/5, 26/6, 5/7, 12/7		
		16/6, 29/6, 5/7, 19/7, 27/7		
	Carbaryl	17/6, 12/7		
2008Mi	neral oil + Fluvalinate	7/3		
	Methomyl	7/4, 4/7		
	Deltamethrin	4/7		
	Thiacloprid	7/5		
	Triflumuron	31/5		
		0.10		

 Table 1. Insecticides and fungicides used in orchard A in the years 2003-2008.

Clausenia purpurea Ishii, an effective CMB parasitoid, *Anagyrus* nr. *pseudococci* (Girault) and *Acerophagus maculipennis* (Mercet) (Hymenoptera Encyrtidae) (Guerrieri and Pellizzari, 2009).

Materials and methods

Phenology and ethology of P. comstocki

The phenology and ethology of *P. comstocki* were studied both in screenhouse and in the field from September 2006 to December 2008. Detailed observations were carried out on artificially infested pot plants in a screenhouse located in the experimental farm of the Agricultural Faculty, University of Padua, Italy (Latitude 45°20'50"N, 11°57'35"E). Two peach trees, two apple trees, one pear tree and one mulberry tree, each about

160 cm high, were infested with masses of *P. comstocki* overwintering eggs, collected in a peach orchard, respectively in November 2006 and November 2007. Observations were carried out every one-two weeks from March to December to assess the pest phenology. Data on 2^{nd} and 3^{rd} instar nymphs were assembled due to the difficulty to separate these instars by visual observation. Data on temperature and rainfall were provided by the local weather station.

Monthly observations were carried out in a highly infested peach and nectarine orchard (orchard A) located at Alpo di Villafranca (Verona district, 45°23'28.59"N, 10°55'56.98"E). Orchard A consisted of 2.5 ha with about 1500 trees/ha planted in 1993; it had a permanent natural cover and was surrounded by a kiwifruit orchard on the northern side, a meadow on the western side and other peach orchards on the remaining sides. It was managed according to conventional cultivation practices. The list of pesticides (fungicides and insecticides) used in the orchard from 2004 till 2008 is reported in table 1.

Distribution patterns of CMB in the study area and in the orchard A

A survey was carried out in the area surrounding the first recognised CMB focus (orchard A) from early June to late August 2008 to assess the distribution pattern of the pest in this fruit growing area. Sampling plots each of 2.5 \times 2.5 km were outlined using Google Earth[®], forming a 10×15 km regular sampling grid of 30 plots, totalling 150 km². Inside each plot, monitoring focused mainly on mulberry trees, because of their broad distribution in the countryside and their suitability for CMB; ornamentals (e.g. Viburnum tinus, Prunus laurocerasus) were monitored when mulberry or peach trees were absent or rare within a plot. Sampling was carried out on six-seven plants/plot. Additional records outside the sampling grid came from inspections performed by the Quarantine Regional Service till 2011. All the specimens were slide mounted and then identified at the compound microscope following the keys of Williams and Granara de Willink (1992) and Kosztarab (1996).

The spatial distribution of CMB in orchard A was assessed in summer 2008 by visual monitoring of one linear meter of branches on each plant of the orchard and assessing the infestation level according to three infestation classes, where: 0 when no mealybug was observed; 1 to moderately infested plants (1-50 individuals/m); 2 to highly infested plants (> 50 individuals/m). Data were analyzed using Spatial Analysis by Distance IndicEs (SADIE) to map the spatial distribution patterns of P. comstocki observed on plants. For each plant we assessed the local contribution to a cluster, that is an area characterized by either plants relatively with high infestation (i.e. a patch) or plants with a relatively low or no infestation (i.e. a gap) close to one another. Tests of non-randomness based on the overall index of aggregation (I_a) and on the average indices of patchiness (\overline{v}_i) and gap (\bar{v}_j) were performed ($\alpha = 0.05$) (Perry *et al.*, 1999). When the overall index of aggregation (I_a) values is equal to 1 indicate random distribution, while $I_a > 1$

indicates aggregation into clusters (Perry, 1995). The average indices of patchiness (\overline{v}_i) and gap (\overline{v}_j) provide further information on spatial pattern detecting the presence of clustering into gaps and into patch in the spatial pattern arrangement (Perry *et al.*, 1999). Using kriging with SURFER (Golden Software Inc., CO), indices of local aggregation (v_i ; v_j) were mapped in a two-dimensional map showing their distribution in the orchard.

Results

Phenology of P. comstocki in screenhouse

The absolute minimum temperatures in winter reached -3 °C in February 2007, and -5.4 °C in February 2008. Apparently they did not affect the overwintering egg masses.

Spring temperatures in the two experimental seasons differed greatly. The average of maximum temperatures in April 2007 was rather high, 22.3 °C, whereas it was only 17 °C in April 2008; moreover, the monthly average of minimum temperatures in April 2007 was 9.6 °C, whereas it was 7.7 °C in April 2008 (table 2).

In 2007 overwintering egg hatching started on April 9th and ended by April 30th; crawlers settled on young leaf undersides and to a lesser extent on apple trees flower buds. By the end of May the population consisted of 2nd and 3rd instars. Female nymphs were recorded on leaves until the beginning of June when first adult females were noticed. After fertilization they secreted a white waxy egg-sac. Egg-laying lasted from mid-June to mid-July. Egg hatching of the second brood occurred from early

July onward and adult females of this generation appeared by late July. Egg-laying started on August 10th and was prolonged for about one month. The first crawlers of the 3rd generation were noticed in late August and the first adult females in the first half of October. By mid-November the population consisted mostly of reproductive females, with rare 3rd instars nymphs. Egg-laying lasted from mid-October to early December. The 3rd generation eggs overwintered. Rare nymphs or adult females were also recorded during winter (figure 1).

In 2008, egg hatching started on April 28th and lasted a week; the last settled 1st instars were noticed in early June, whereas on mid-June the nymph population consisted mostly of 3rd instars. The first adult females were observed in middle June, whereas in late June the whole population consisted of adult females. Egg-laying lasted from late June to mid-July. Egg hatched in July and the 1st instars were observed until late July. The first adult females of 2nd generation were observed from early August onward. They laid eggs from mid-August to mid-September. Crawlers occurred from late August to early October. The first adult females of 3rd generation were observed in early October, whereas egg-laying lasted from mid-October to mid-November. Some rare nymphs and egg-laying females were still alive in December (figure 1).

Observations carried out in orchard A confirmed this trend. The adult females were recorded respectively in June-July (1st generation), in August-September (2nd generation), from October onward (3rd generation). According to these data, in the Veneto Region, CMB completes three distinct generations per year (figure 1).

Month	Year 2007			Year 2008		
	T min	T max	Average T	T min	T max	Average T
January	2.6	9	5.6	1.6	8.5	4.7
February	3	11.5	6.9	0.7	9.8	4.9
March	5.6	15.3	10.4	4.1	12.9	8.2
April	9.6	22.3	16.1	7.7	17.1	12.5
May	13.8	24.5	19.1	12.9	22.6	18
June	16.5	27.1	22.1	16.2	26	21.1
July	16.2	30.5	23.7	18.3	29.6	23.9
August	16.1	27.7	21.9	17.7	29.3	23.4
September	11.9	23.3	17.4	13.3	23.5	18
October	8.9	18.5	13.3	10	20.5	14.6
November	3.3	12.4	7.5	5.9	12.4	8.8
December	-0.2	7.7	3.2	2	7.6	4.7

Table 2. Monthly average of minimum, maximum and medium temperatures during 2007 and 2008.

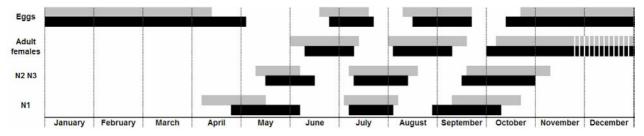


Figure 1. Phenology of *P.comstocki* in the years 2007 (grey line) and 2008 (black line). The dotted lines indicate that rare females were observed till December.

Ethology of P. comstocki

CMB crawlers colonize the leaf lower surface, mainly localized near the main veins. Female nymphs feed and moult on leaves, whereas 2nd instar male nymphs mostly move from leaves searching for hiding places where they secrete a cocoon and complete their development. Adult males have a very short life span and thus they are rarely encountered in the field. CMB 3rd instar nymphs and mostly adult females demonstrate a clear tendency to move towards branches and trunk searching for sheltered places to lay eggs. Females often congregate on pruning scars and nodes of branches, into crevices, under the bark where they produce a white waxy egg-sac. When high infestations take place, masses of white egg-sacs are clearly visible on trunks and old branches. Moreover, adult females may fall from leaves to ground, and this behaviour allows their passive dispersal. Many egg-sacs have been detected on dried leaves fallen on the ground. On young trees or shrubs with smooth bark, females move to the ground. Searching for sheltered places the females infest fruits, localizing in the stem end of peaches and in the calyx cavity on apples. The tendency to move from leaves to trunks, branches, fruits or other protected places increases in the 2^{nd} and 3^{rd} generation adult females with respect to the 1^{st} generation adult females.

In orchard A, infested ripe nectarines, with 3rd instars and adult females concealed in the stem end, are picked up by the end of July-early August, whereas peaches are picked up about a month later. Adult females were observed while actively moving from the picked fruits to the walls of the plastic fruit-bins ready for consignment. This way the CMB can be easily transported far away with trading.

CMB is strongly attended by ants that protect it from natural enemies and spread mealybugs around. In orchard A ants were observed while transporting mealybugs from branches to the crown zone of the trunk, at ground level, where surface roots radiate out from the tree trunk, and a protective soil nest is built all around the infested roots.

Table 3. CMB host plants and locations in the Veneto Region. An asterisk marks the new CMB host plants recorded during the survey.

H	ost plants	Location	District	Year
Anacardiaceae	Cotinus sp.*	Treviso	Treviso	2008
Araliaceae	Hedera helix*	Treviso	Treviso	2008
Berberidaceae	Mahonia aquifolium*	Treviso	Treviso	2008
Betulaceae	Carpinus betulus*	Treviso	Treviso	2008
Bignoniaceae	Catalpa bignonioides	Castelnuovo del Garda	Verona	2007
Caprifoliaceae	Viburnum tinus	Treviso	Treviso	2006, 2008
		S. Martino Buon Albergo	Verona	2007
		Ca di David	Verona	2008
		Verona	Verona	2008
Elaeagnaceae	Elaeagnus sp.*	Treviso	Treviso	2008
Hypericaceae	Hypericum calycinum*	Castelnuovo del Garda	Verona	2006
Moraceae	Morus alba	Verona	Verona	2004
		Dossobuono	Verona	2008
Moraceae	Morus nigra	Dossobuono	Verona	2008, 2009
		Rizza	Verona	2008
		Castel d'Azzano	Verona	2008
		Alpo, Ca di David	Verona	2008
		Marchesino Bovo	Verona	2008
		Verona	Verona	2008
		Paese	Treviso	2011
Moraceae	Morus pendula	Castelnuovo del Garda	Verona	2009
Pittosporaceae	Pittosporum tobira	Treviso	Treviso	2008
Rosaceae	Cotoneaster horizontalis*	Treviso	Treviso	2008
Rosaceae	Malus communis	Legnaro	Padova	2007, 2008, 2011
		Ronco all'Adige	Verona	2012
Rosaceae	Prunus persica	Alpo di Villafranca	Verona	2006, 2007, 2008, 2009
		Dossobuono	Verona	2007, 2008, 2009
		Lugagnano-Sona	Verona	2008
		Verona- Dieci Bine	Verona	2008
		Buttapietra	Verona	2008
		Treviso	Treviso	2010
Rosaceae	Prunus laurocerasus*	Treviso	Treviso	2006, 2008
		Villafranca	Verona	2008
Rosaceae	Pyracantha coccinea*	Treviso	Treviso	2008
Rosaceae	Pyrus communis	Legnaro	Padova	2007, 2008
		Cavarzere	Venezia	2012

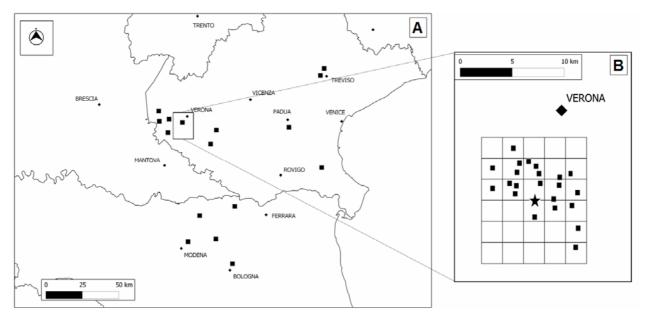


Figure 2. A: Distribution map of *P. comstocki* in northeastern Italy: black squares indicate infested sites; B: presence of CMB in the sampling grid area around the first recognized CMB focus (orchard A, black star): black squares mark the infested foci recognized during the survey.

Mealybugs feed on roots, females lay eggs and nymphs develop in a totally sheltered place, escaping from any visual observations and insecticide applications.

Host plants

In the Veneto countryside, CMB was mostly recorded off peach and mulberry trees, whereas in urban environments the mealybug infested several ornamentals. A list of CMB host plants and locations is provided in table 3. Among them *Cotinus* sp. (Anacardiaceae), *Hedera helix* (Araliaceae), *Mahonia aquifolium* (Berberidaceae), *Carpinus betulus* (Betulaceae), *Elaeagnus* sp. (Elaeagnaceae), *Hypericum calycinum* (Hypericaceae), *Cotoneaster horizontalis, Prunus laurocerasus, Pyracantha coccinea* (Rosaceae) proved to be new host plants of *P. comstocki*.

Distribution pattern of CMB in the study area

The presence of CMB in the area surrounding the orchard A was remarkable. Infested mulberry and peach trees or infested ornamentals were recorded in 16 plots out of 30, over an area of about 100 km², placed around the first detected focus (figure 2). Outside the sampling grid, CMB was detected in several locations in the districts of Verona and Treviso, mostly on ornamentals, in 2010 it was recorded for the first time in a peach orchard in the Treviso district, whereas in 2012 heavy infested organic apple and pear orchards were detected in Verona and Venice districts.

Distribution pattern of CMB in orchard A

Distribution pattern of CMB in orchard A showed a clear aggregation within the orchard ($I_a = 3.23$; P = 0.0008). Spatial pattern was characterized by significant clustering into patches ($\overline{v}_i = 2.83$; P < 0.0001) as well as gaps ($\overline{v}_j = -3.28$; P < 0.0001). Map of local aggregation indices evidenced a presence of a patch in the centre of

the orchard, while gaps were along the borders except the south-western corner (figure 3).

Damage

Nymphs and young adult females excrete a huge amount of honeydew that drops on leaves and fruits below on which usually a dense sooty mould develops. Ornamentals assume an unsightly appearance: the foliage turns to dirty blackish; early leaf fall was observed on highly infested *Morus*, *P. laurocerasus* and *Viburnum* plants. On mulberry, colonies of mealybugs cause gall formation on young twigs. On apple trees the 1st generation nymphs can infest the flower buds. As a consequence the flowers wither and the young fruits result misshapen and stop their growth. Nymphs and adult females often invade the calyx and fruit-stem cavities of peach, apple and pear fruits. This may considerable depreciate fruits, especially when associated with sooty mould development on excreted honeydew.

Discussion

In northeastern Italy CMB develops three generations per year and overwinters in the egg stage (figure 1). Egg-sacs are produced in protected places, under bark or in bark crevices, on trunk and large branches, on dried leaves fallen on the ground. Both in 2007 and 2008 egg hatching occurred in April, even if at different dates, depending on climatic conditions and phenology of the host plant. The unusual high temperatures recorded in spring 2007 were associated to an early egg hatching (20 days) with respect to 2008. April 2007 was sunny, with only one rainy day whereas April 2008 was unusually overcast and rainy, with 13 rainy days. However, starting from the 2nd generation onwards, the phenology in 2008 was quite similar to that observed in 2007. The adult females of the three

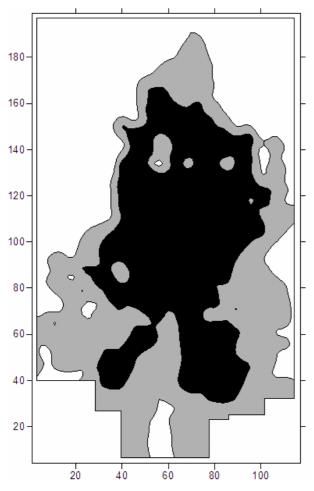


Figure3. Map of local aggregation of *P. comstocki* observed inside the peach orchard A in 2008. Within the orchard black areas are patches, with interpolated cluster index, $v_i > 1.5$, white areas are gaps with interpolated cluster index $v_j < -1.5$. Values on axis indicate coordinates in meters.

generations occurred mainly in June-early July (1st generation); from late July to half September (2nd generation); from late September until November (3rd generation), with rare females still alive in December. The phenology of CMB in the Veneto Region is very similar to the one reported in Virginia, USA (Hough, 1925). During May of both seasons CMB population consisted mostly of 1st and 2nd instars settled on the leaf undersurface. Chemical control should be performed in this period as the most susceptible stages are exposed to pesticides.

In orchard A, CMB appeared to be aggregated in the central area whereas borders were much less or not infested. This distribution could be affected by the occurrence of antagonists, mainly parasitoids, in the crop borders. The occurrence of CMB encyrtids in the monitored area (Guerrieri and Pellizzari, 2009) could be involved in the distribution pattern of the pest. In the centre of the orchard repeated treatments with deltamethrin carried out in the previous years could have negatively affected parasitoid and predator populations.

CMB is widely distributed in the Veneto Region, in fruit orchards and on ornamentals. The recent findings in organic orchards and in the neighbouring EmiliaRomagna Region (Masi *et al.*, 2010; Reggiani *et al.*, 2011) indicate that this species is expanding its distribution in Italy. The results of monitoring highlight that mulberry trees host quite often CMB populations. Mulberry trees are historically widely distributed in the Venetian countryside; they often form rows along fields, or grow as shadow trees near farmhouses. These infested trees act as permanent sources of CMB from where the mealybugs can spread to the contiguous cultivated areas, mainly fruit orchards. CMB nymphs and adult females demonstrate to be highly mobile, moreover they conceal themselves in the fruit calyx and stem end. The movements of fruit bins from an orchard to another at the picking time and the fruit trade point out on the possible further spreading of this pest.

In the meantime mulberries could act as parasitoid reservoir. The presence of native and alien parasitoids such as A. maculipennis, Anagyrus sp. near pseudococci and, above all, C. purpurea, in the Verona district (Guerrieri and Pellizzari, 2009), is a promising perspective of sustainable control of the pest. C. purpurea is a well-known parasitoid of CMB in Japan and was introduced for biological control against CMB in California (Murakami et al., 1967; Bartlett and Clancy, 1972; Meyerdirk and Newell, 1979a) as well as against Pseudococcus cryptus Hempel in Israel (Blumberg et al., 1999). Very recently, C. purpurea was also recorded from infested mulberry trees in France (Fleisch et al., 2011). Since no parasitoid releases were made in Europe, we can assume that C. purpurea was introduced along with its host. This possibility was also suggested by Clancy (1944) who observed that in Virginia C. purpurea occurred wherever the CMB was found. It is also worth mentioning the predaceous activity of Harmonia axyridis (Pallas), an Asiatic invasive coccinellid recently established in northern Italy (Burgio et al., 2008) and recorded in CMB infested pear orchards (Masi et al., 2010).

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