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Biology and seasonal fluctuation of cottony camellia scale, *Pulvinaria* (*Chloropulvinaria*) *floccifera* (Hemiptera: Coccidae) in Citrus orchards of northern Iran

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Abstract

Cottony camellia scale, *Pulvinaria floccifera* Westwood is almost cosmopolitan having been spread widely by the plant trade. This soft scale is one of the most important scales of citrus orchards in Mazandaran and Guilan provinces. The biology and seasonal fluctuation of pest was studied in natural conditions in the West of Mazandaran. Furthermore, the biology of this soft scale was studied in laboratory condition. To study population dynamics, 10 leaves from each five Thomson navel orange trees, *Citrus sinensis* L. were taken every two weeks. *P. floccifera* completed one generation per year, hibernating as an adult insect. The last overwintering females were observed in early June and Middle May in 2009 and 2010, respectively. First instar nymphs appeared in middle July and late June. Second instar nymphs were first observed in early August and middle July, peaked in last August and last July in 2009 and 2010, respectively, whereas the third instar nymphs peaked in late September and late August. The complete life cycle of females required 58.2±2.1 days in the laboratory conditions, 25±10ºC, 70-80% RH, and 14:10 (L:D). Number of eggs in egg sac ranged from 445 to 680. Data showed that the ladybirds *Cryptolaemus montrouzieri* Mulsant and *Chilocorus bipustulatus* Gordon feed on *P. floccifera*. This study revealed that *P. floccifera* is present in all citrus plants sampled in the area of study in Mazandaran and Guilan provinces.

Keywords: cottony camellia scale, seasonal population dynamics, Citrus fruits

Introduction

The genus *Pulvinaria* (Sternorrhyncha: Coccidae) contains more than 100 described species (Hodgson, 1994; Ben-Dov, 2009) of which 25 species have been recorded from the New World (Ben-Dov *et al*., 2009). The cottony camellia scale, *Pulvinaria floccifera* (Westwood, 1870) is a species common in the Mediterranean region that has a world-wide distribution (Kosztarab & Kozar, 1988; Ulgenturk *et al*., 2004). It is a polyphagous species associated with host plants from at least 34 families (Ben-Dov *et al*., 2008), most frequently found on the leaves and branches of *Pittosporum* spp., *Euonymus* spp., *Ilex* spp., *Mahonia* spp. and *Taxus* spp. (Milek *et al*., 2009). Feeding on leaves and twigs by nymphs and adult females results in production of large amounts of honeydew that encourages the growth of black sooty mold and can weaken the host plant, causing leaf loss and slow dieback of twigs and branches. Soft scales might be a vector of plant

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viruses, for example, *P. vitis* L. can transmit the economically important grapevine leaf roll virus (Elborolosy *et al*., 1990).

There is clear evidence that climate change is altering the distribution, incidence and intensity of animal and plant pests (www.ftp.fao.org; Rasekh *et al*., 2011). For example, it has been reported a dramatic increase in enquires regarding to *P. floccifera*. This pest is frequently intercepted on imported plants, most often on Camellia from France, Italy, Netherlands and New Zealand. For many years this soft scale was restricted to Camellia and South of England but has now spread throughout England and has also been recoded on *Choisya*, *Citrus*, *Juniperus*, *Kalmia*, *Laurus*, *Pieris*, *Pyracantha*, *Magnolia*, *Rhododendron* and *Trachelospermum*. So, over the last decades, this insect has become a more damaging pest (www.fera.defra.gov.uk). In Sweden this species was previously only known as a greenhouse species, but is now established as an outdoor species (Gertsson, 2005).

In 1951, Cottony camellia scale was considered as one of the most important pest of citrus trees in Egypt. A study on biology of this species showed that female fecundity and generation time was 875 eggs and 134.4 days. Furthermore, the unmated females produce female offspring (Elborolosy *et al*., 1990). In a study on spatial distribution of citrus whitefly, *Dialeurodes citri* (Ashmead) and *P. floccifera,* it has been shown that these two pests had the simultaneous peak abundance in Palestine (Ben-Dov *et al*., 2001). The effect of season on growth rate and fecundity of *P. psidi* and *P. floccifera* was studied in Spain (Helyer *et al*., 2003).

Biology of *P. regalis* in laboratory and natural conditions was investigated, and the sizes of anal plates were used to distinguish different growth stages (Grafton-Cardwell, 2000). Another study on this pest in natural condition showed that larger females had more fecundity and lager eggs (Ben-Dov *et al*., 2001).

Citrus fruits are the most important orchard production in Mazandaran province with 90000 hectares and yearly production of 1600000 tones. *P. floccifera* is the most harmful soft scale of citrus orchards in Mazandaran and Guilan provinces. Therefore, in this study, biology, local distribution, seasonal population dynamics and natural enemies of the cottony camellia scale were investigated in natural conditions in west of Mazandaran province. Furthermore, local distribution of the pest was investigated in east of Guilan province. The biology of this soft scale was also studied in laboratory condition.

Material and Methods

Study location

The area of study is located between the west of Mazandaran province (Chalous city) and the east of Guilan province (Langroud city), in south coast of Caspian Sea in north of Iran.

Laboratory study

Citrus leaves bearing *P. floccifera* were collected from citrus orchards in the area of study. A total of 50 individuals of each representative of a life stage were examined and measured in order to characterize distinguishing features.

Two or three year-old potted of sour orange, *Citrus aurantium*, L. (n= 10) held in a growth chamber at 25±1ºC, 70-80% RH, and 14:10 (L:D) photoperiod. Infestation was accomplished by placing a leaf with egg sacs (n=15) onto each sapling for a period of 10 days. After the crawlers had settled, 200 nymphs on each sapling were retained and the rest were removed. Every two days, five soft scales on each sapling were dissected randomly under stereomicroscope. The lengths of anal plate were used to distinguish each instar nymph. Sexes can be distinguished after the second nymphal instar.

In order to estimate female egg load, we collected infested leaves from sour orange, *C. aurantium*, in west of Ramsar in early July 2009, and transported them to the laboratory. Egg sacs (n= 30) were each placed in an 11×1 cm petri dish. In order to solve the wax cover of egg sac to count the number of eggs, they were washed by droplets of alcohol.

Field study

The distribution of *P. floccifera* in the area of study was determined by inspecting potential host plants in various locations from middle May to early December, 2009 and 2010. A minimum of 5 trees of each plant species were inspected at each location of one kilometer apart.

According to method of Uygun and *et al*. (1995), 10 leaves from each five Thomson navel orange trees, *Citrus sinensis* L. in an infested orchard in west of Ramsar were sampled randomly, every two week from May to November 2009 and 2010. Leaves were taken to the laboratory and all insects were dissected under a stereomicroscope to determine their growth stages. These data were used to study the seasonal population dynamics of the pest.

From April to October 2009 and 2010, many ladybird species were collected in citrus orchards in local distribution. Adult ladybirds collected of various species were introduced in an 11×1 cm petri dish containing immature stages of soft scales separately to determine if the beetles feed on them.

Results

Laboratory study

The newly emerged first instar nymphs or crawlers have milky or yellowish light color, oval in shape, with antennae and three pairs of legs. These crawlers disperse over the plant and may be carried over longer distances by wind. After settling, the legs were hidden under body. This newly eclosed nymphs settle under and above the leaves within 4-24 h and then migrate to stem as second instars. Crawlers settle on leaves while later instars settle on leaves and green branches, where they mature. Anal plate of first instar nymphs averaged 0.04±0.003 mm in length. The second instar nymphs are oval, darker and bigger in comparison to the first instar. Anal plate of second instar nymphs averaged 0.07±0.002 mm in length. The third instar nymph is yellow or yellowish brown, which its color changes gradually to dark yellowish. The body is wider and more convex than the second instar nymph. Anus plate is brown with the mean of 0.1±0.06 mm in length.

Adult females are 3–4.5 mm long, 2.0-3.0 mm wide, and body elongate oval, fairly flat in cross section, somewhat transversely ridged and dark brown, without obvious wax covering. They are mainly on the shoots. Young adult females are brown, usually with a yellow medial stripe. The anal plate averages 0.15±0.05 mm in length. The end of adult female's body is separated from leaf surface during oviposition. Ovisac is produced beneath and behind of the female body, elongate, relatively flat, white, flocculent, about two times the length of body (4-9 mm). The size of egg sac does not change since the eighth day, but the egg laying will be continued. Ovisac is waxy that resembles cotton wool. Eggs are laid inside ovisac and the latest eggs will be laid on the top of egg sac. The ovisacs are attached to underside of leaves. During oviposition on vertical branches, the head of female is placed towards the main branch. The adult female dies at the end of oviposition period and its body dries up as a brown and hard skeleton.

The spiracles can be seen in nymphal instar and young adult female but they disappear in mature adult female. Adult males have been explained by Canard (1969).

The mean durations of immature stages and total developmental times for female insects has been presented under laboratory conditions. The life cycle of female insects obtained 58.2±2.1 days (Table 1).

Counting the eggs in egg sacs collected from infested branches revealed the egg loads ranging from 445 to 680 eggs (mean= 560±78).

Table 1- Mean (±SEM) developmental time (in days) of immature stages of *P. floccifera* female raised (n=50) on potted sour orange under laboratory conditions, 25±1 ^{0}C, 70-80% RH, and 14:10 (L: D) photoperiod

Growth stages	Min.	Max.	Mean ± SE
Embryon	10	12	11.1 ± 0.7
First nymphal instars	12	14	13.6 ± 0.9
Second nymphal instars	14	16	15.0 ± 1.2
Third nymphal instars	17	20	18.7 ± 1.3
The life cycle of female insects	56	61	58.2 ± 2.1

Field study

The study revealed that *P. floccifera* was present in all sampling regions of Mazandaran and Guilan provinces (Table 2). Observations revealed that orchards in Langroud and Abbas abad were more infested than other regions.

Pulvinaria floccifera has one generation per year and hibernates as an adult insect. The last overwintering females were observed in early June and Middle May in 2009 and 2010, respectively (Figs. 1 & 2). First instar nymphs appeared in middle July and late June, peaked in early August and middle July, and disappeared by early September and late August in 2009 and 2010, respectively. Second instar nymphs were first observed in early August and middle July, peaked in last August and last July, whereas third instar nymphs peaked in late September and late August, and disappeared in middle November and middle October in 2009 and 2010, respectively. The overwintering adult insects appeared in late October and middle October (Figs. 1 & 2).

Larval and adult forms of the following coccinellids species were collected from citrus orchards in the area of study; *Cryptolaemus montrouzieri* Muls, *Chilocorus bipustulatus* Gordon, *Oenopia conglobata* L. and *Serangium montazeri*, while only *C. montrouzieri* and *C. bipustulatus* feed on all stages of *P. floccifera*.

Table 2- Locations and tree species inspected for *P. floccifera* raised in Mazandaran and Guilan provinces, Iran, in 2009-2010

Province	Region	Elevation	Tree species
Mazandaran	Ramsar (Sefidtamashk, Sadatshahr & Katalom)	8	*Camellia sinensis, Citrus aurantium, C. sinensis*
	Tonekabon (Khoram abad & Zangsha mahaleh)	-20	*Citrus paradise, Citrus limmettioides*
	Salman Shahr (Danial)	3	*Citrus sinensis, Citrus aurantium*
	Chalous (Zavat)	0	*Citrus sinensis*
	Abbas abad (Asb chin & Hasandeh)	2	*Citrus sinensis, Citrus aurantium*
	Nashtaroud (Dinarsara & Polsara)	-18	*Citrus sinensis*
Guilan	Amlash (Esmail govabar, Kohneh gooyeh, Abbas govabar & Otaghvar)	-5	*Camellia sinensis*, *Citrus aurantium*
	Kelachay (Hasanvareh, Ghasem abad & Reza mahaleh)	-20	*Citrus limmettioides*
	Langroud (Leilakooh, Parshkooh & Malat)	21	*Citrus aurantium, Citrus sinensis, Camellia sinensis*
	Chaboksar (Miandeh)	-26	*Citrus aurantium*, *Citrus paradise*

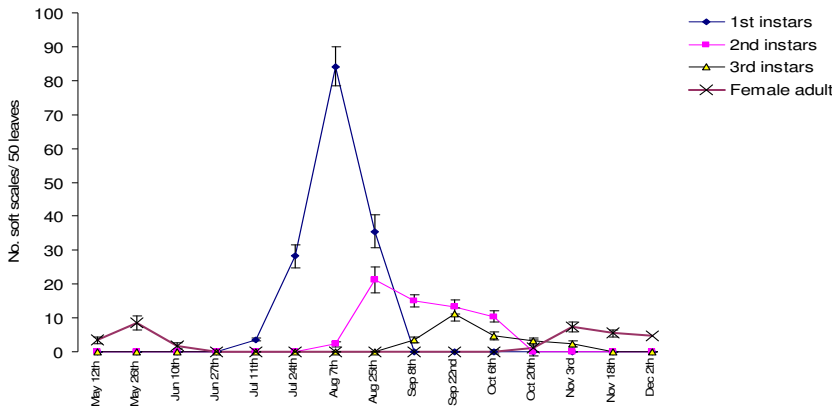

Fig 1. Total number of different developmental stages of *Pulvinaria floccifera* on 50 leaves of 5 Thomson navel orange trees in West of Ramsar, Iran, 2009

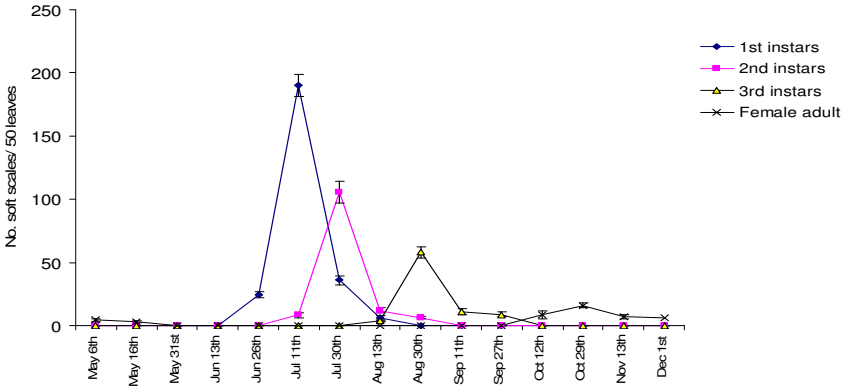

Fig 2. Total number of different developmental stages of *P. floccifera* on 50 leaves of 5 Thomson navel orange trees in West of Ramsar, Iran, 2010

Discussion

Pulvinaria floccifera completes one generation per year in the area of study, hibernating as an adult insect. In a study in Croatia, overwintering stage has been as the second nymphal instar (Milek *et al*., 2009).The female overwinters on stems and migrates to leaves in spring where an ovisac is produced for egg deposition. The numbers of eggs in egg sacs collected from infested branches were determined in laboratory condition 560±78 eggs, while in a study in Egypt; it has been obtained 857 eggs (Elborolosy *et al*., 1990). These authors determined the hatching of eggs 23 days, in 17-20°C while this period was 5 days in constant temperature, 27°C. Furthermore, the mean durations of 1st, 2nd and 3rd instar nymphs of *P. floccifera* in 22.5°C were 11, 16 and 10 days respectively. These durations in 27°C were obtained 19, 10 and 14 days respectively (Elborolosy *et al*., 1990).

In this research crawlers appear in late June or middle July and they are active till late August or early September in different years of sampling, depending on environmental conditions. The last crawlers are produced by females that have emerged in late June. According to the results, the crawlers, the most sensitive stage of the pest to insecticide applications, appear in a long period, and this makes it difficult to act against them. In a study on using Growing Degree-Days (GDD) for Insect Pest Management, range of GDD for crawlers of *P. floccifera* were determined 802-1388 (www.fera.defra.gov.uk).

Seasonal population dynamics of *P. floccifera* in 2009 and 2010 display some difference. First instar nymphs peaked at the beginning of August and in the middle of June respectively (Table 1

& 2). It seems that different environmental condition especially the higher temperature in study location in 2010, according to meteorological office data, caused these variations.

Pulvinaria floccifera is highly polyphagous. In this study, the pest was found on *Camellia sinensis* and various species of the genus *citrus*, but the most numerous populations have so far been found on *Taxus baccata*, *Pittosporum toriba* and on *Ilex aquifolia* (www.entocare.nl; Seljak, 2008). During a research in Croatia, *P. floccifera* was found on seven host plants *Ilex aquifolium* L., *Aralia japonica* Thunb*., Mahonia aquifolium* (Pursh) Nutt., *Euonymus japonicus* Thunb., *Aucuba japonica* Thunb., *Pittosporum tobira* Ait. and *Taxus baccata* L. which belong to seven plant families, on 17 localities, including Mediterranean and continental part of Croatia (Milek *et al*., 2009).

Our observations showed that ladybirds *C. montrouzieri* and *C. bipustulatus* are active as predators in citrus orchards. In other studies, ladybirds *C. montrouzieri* (Prokopenko *et al*., 1982; Helyer *et al*., 2003) *Scymnus coccivora* Ayyar, *Chilocorus stigma* Say (Ben-dov *et al*., 2001) *C. bipustulatus* L. and *Exochomus quadripustulatus* L. (Milek *et al*., 2009) *Coccophagus lycimnia* (Walker) (Peck, 1963; Milek *et al*., 2009), *Metaphycus swirskii* (Japoshvili & Celik., 2010) are reported as predators of *P. floccifera*.

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بیولوژی و نوسانات فصلی شپشک کاملیا *Pulvinaria* (*Chloropulvinaria*) *floccifera* (Hemiptera: Coccidae) در باغ‌های مرکبات شمال ایران

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چکیده

بالشتک مرکبات *Pulvinaria floccifera Westwood* یک آفت جهانی بوده و همراه با تجارت گیاه به‌طور وسیعی گسترش یافته است. این گونه یکی از مهمترین بالشتک‌ها در باغ‌های مرکبات در استان‌های مازندران و گیلان می‌باشد. زیست‌شناسی و تغییرات جمعیت آفت در شرایط طبیعی غرب مازندران طی سال‌های ۱۳۸۸ و ۱۳۸۹ مطالعه شد. همچنین زیست‌شناسی این آفت در شرایط آزمایشگاه بررسی گردید. به‌منظور مطالعه تغییرات جمعیت بالشتک، ۵ درخت تامسون ناول، *Citrus sinensis L.* در یک باغ در رامسر انتخاب و هر دو هفته یک‌بار، ده برگ از هر درخت نمونه‌برداری شد. نتایج نشان داد بالشتک مرکبات در منطقه مورد مطالعه یک نسل در سال دارد و زمستانگذرانی ماده‌ها به‌صورت حشرات کامل می‌باشد. آخرین حشرات زمستانگذران به‌ترتیب در سال‌های ۱۳۸۸ و ۱۳۸۹ در اواسط خرداد و اواخر اردیبهشت مشاهده شدند. پوره‌های سن اول به‌ترتیب سال‌های مطالعه در اواخر و اوایل تیر ظاهر گردیدند. پوره‌های سن دوم در اوایل شهریور و اوایل مرداد به اوج جمعیت رسیدند. این اوج جمعیت در پوره‌های سن سوم به‌ترتیب سال‌های مطالعه، در اواخر و اوایل شهریور دیده شد. حشرات زمستان‌گذران در اواخر و اواسط مهر ظاهر شدند. دوره کامل زندگی حشرات ماده در شرایط آزمایشگاهی (۱± ۲۵ درجه سلسیوس، رطوبت نسبی ۵±۷۵ درصد و دوره نوری ۱۶ ساعت روشنایی و ۱۰ ساعت تاریکی) ۲/۱±۵۸/۲ روز طول کشید. میانگین تعداد تخم موجود در کیسه‌های تخم ۷۸±۵۶۰ تعیین شد. دو گونه کفشدوزک *Cryptolaemus montrouzieri Mulsant* و *Chilocorus bipustulatus Gordon* روی بالشتک مرکبات در تمام مناطق نمونه‌برداری شده در این دو استان شمالی کشور مشاهده شدند

واژه‌های کلیدی: شپشک کاملیا، تغییرات فصلی جمعیت، مرکبات

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