

Population changes of *Pulvinaria aurantii* and its predatory ladybird *Cryptolaemus montrouzieri* in Tonekabon citrus groves

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ARTICLE INFO ABSTRACT Article history: The orange pulvinaria scale, Pulvinaria aurantii Cockerell (Hemiptera: Coccidae), Received: 24 April 2022 is one of the most significant citrus orchard pests in northern Iran. To assess the Accepted: 29 May 2022 population fluctuations of this pest and its predator Cryptolaemus montrouzieri Available online: 1 June 2022 Mulsant in Tonekabon blood orange orchards, 80 leaves from 20 trees were Keywords: randomly selected at each sampling time (with a maximum relative variation of Blood orange 15%) and the number of each biological stage of the P. aurantii and its predatory Lady beetle ladybird were recorded per leaf. The average population of all biological stages of Orange pulvinaria scale P. aurantii (ovisac, nymphal stages, and adult female insect) peaked on June 29 Overlap (30.05 per leaf) and September 14 (29.55 per leaf) in 2011, and on June 21 (30.09 Population dynamics per leaf) and September 6 (22.6 per leaf) in 2012. Similarly, the average population of all C. montrouzieri biological stages peaked on June 29 (0.34 per leaf), September 7 and 14 (0.45 per leaf), and June 21 (0.65 per leaf) and September 6 (1.00 per leaf) in 2011 and 2012, respectively. The population change curves indicated that increasing the population of ovisacs, 1st and 2nd instar nymphs of the pest attracted and increased the population of C. montrouzieri on infected trees, possibly due to the desirability of these biological stages of the pest to the predator. The present study revealed that in both years, the second generation of the pest is characterized by greater concordance and overlap between the populations of scale ovisacs and ladybird eggs. In 2011, C. montrouzieri prevented an increase in the population of *P. aurantii* second generation, and in 2012, due to a higher population density, it was able to significantly reduce the second generation population of this soft scale. Additionally, the regression between prey and predator populations was statistically significant, indicating a density-dependent response of the predator to the prey population.

Highlights

- Pulvinaria aurantii Cockerell is a major citrus orchard pest in northern Iran.
- The average population of *P. aurantii* peaked on June 29 (30.05 per leaf) and September 14 (29.55 per leaf) in 2011, and on June 21 (30.09 per leaf) and September 6 (22.6 per leaf) in 2012.
- In both years, the second generation of the pest has increased concordance and overlap between scale ovisacs and ladybird eggs.
- In 2011, *C. montrouzieri* prevented a growth in *P. aurantii* second-generation population, and in 2012, it substantially decreased this soft scale's second-generation population.

1. Introduction

Iran is one of the countries where citrus fruits are produced economically. According to FAO statistics, in 2020 the country was ranked 9th in the world in terms of

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citrus production (Anonymous, 2020). The orange pulvinaria scale, *Pulvinaria aurantii* Cockerell (Hemiptera: Coccidae), is one of the most important pests in the north of Iran (Moghaddam, 2010) that weakens the citrus trees by sucking plant sap and causes the growth of sooty mold (*Capnodium citri* Penz.) by the secretion of honeydew, which reduces the quality and marketability of fruits. This insect causes leaf and fruit fall and plant

Email: m.amirkalaee@gmail.com http://dx.doi.org/10.22034/aes.2022.339021.1033

drying in outbreak conditions. (Hallaji Sani, 1999; Damavandian et al., 2014).

The *P. aurantii* female has different life stages, including eggs, nymphs $(1^{st}, 2^{nd}, \text{ and } 3^{rd} \text{ instar})$, and adult insects. In the male insect, the 2^{nd} instar nymph becomes pre-pupae and then pupae. Male insects live for one or two days, and adult female insects live for 12 to 15 days. The oviposition period lasts about 10 days, and the number of eggs in an ovisac of *P. aurantii* on different hosts varies from 350 to 500 (Hallaji Sani, 1999).

The population density of scale insects is affected by temperature, relative humidity, climatic conditions, pesticide application, and biological agents (Ullah, 1992; Hallaji Sani, 1999; Abdel-Moniem, 2003). At present, insecticides are mostly used in the north of Iran to control this pest, as well as other scale insects (Hallaji Sani et al., 2021). Insecticides, in addition to adverse environmental effects, are not always effective and may be washed away by unpredictable rainfall or may not be able to control the pest at all stages (McDowell et al., 1984). In contrast, biological control agents are more stable and reduce the cost of reusing insecticides (van Driesche, 1994; Kimberling, 2004).

Among the natural enemies of this pest, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is a polyphage and native Australian insect that has a higher relative abundance (Bozorg-Amirkalaee et al., 2017) and is widely used in biological control programs of different species of scale insects in the world (Hodek and Honěk, 2009; Kairo et al., 2013; Pérez-Rodrígueza et al., 2019; Ferreira et al., 2021). In Azerbaijan in 1982, researchers released 5,000 *Cryptolaemus* in a three-hectare garden and prevented the spread of *Chloropulvinaria* (Prokopenko et al., 1982).

Wax filaments produced by scale insects stimulate the oviposition of *C. montrouzieri* (Gharizadeh Gelsefidi et al., 2004; Merlin et al., 1996a). The female ladybird usually lays their eggs individually or in groups in the mass of prey eggs, so that the larvae of early ages can easily find their prey (Gharizadeh Gelsefidi et al., 2004). The development time from egg to adult insect of this ladybird is 27.7 days by feeding on *P. aurantii* ovisacs on blood orange, and its oviposition period has been reported to be 70 days (Bozorg-Amirkalaee et al., 2015). The average and maximum number of ladybird eggs per day on *P. aurantii* were 12.5 and 25 eggs, respectively (Gharizadeh Gelsefidi et al., 2004). Feeding of *Cryptolaemus* eggs by conspecific larvae has also been observed in low prey densities (Merlin et al., 1996b).

Population density changes of *P. aurantii* have been studied by Hallaji Sani (1999), Rajabpour (2006), and Damavandian et al. (2014), but no comprehensive research has been done on the population fluctuations of the *Cryptolaemus* predatory ladybird by feeding on different stages of this pest. Therefore, information about population fluctuations of the biological stages of this pest during the growing season as well as its predominant natural enemy, *C. montrouzieri*, will play an effective role in designing integrated pest management.

2. Methods and Materials

This study was conducted in the blood orange orchards of the Tonekabon region, Iran (latitude 36 $^{\circ}$ 70 North and longitude 51 $^{\circ}$ 10 East) in 2011 and 2012.

In the assessment of population changes and in order to reduce the sampling error, the sampling program and the number of samples were first determined. In this study, the statistical population in the field sampling was the orange pulvinaria scale population. Based on the initial studies, blood orange leaves were selected as the sampling unit. To determine the sample size, an initial sampling of 30 leaves was performed on April 25 (2011) by nymphs present at the time. Using the data from the initial sampling, the relative variation (RV), which indicates the accuracy of sampling, was obtained using the Eq. 1:

RV = (SE/m) 100

(1)

where m and SE are the mean density and standard error of the primary sampling data, respectively. Then the sample size (N) was obtained through the Eq. 2:

 $N = [ts/dm]^{2}$

(2)

where m and S are the mean density and standard deviation of the initial sampling data, respectively, d (RV) is the range of accuracy, and t is the numerical value of the student table in terms of the degree of freedom of the sample (Pedigo and Buntin, 1994).

In this study, the number of samples required for the sampling program with a maximum relative variation of 15% was equal to 77 leaves, of which 80 leaves were used as the number of samples required for field studies. With the advent of other stages of *P. aurantii* as well as *C. montrouzieri* ladybird, the RV value was examined for the different biological stages of the pest and its predator (N = 80 leaves) in each season of both studied years during the sampling period. Because the RV value was within the acceptable range, sampling was done with the same number of samples (80 leaves).

To study the population changes of P. aurantii and its predominant natural enemy, C. montrouzieri ladybird, four gardens approximately two kilometers apart were selected in this area. No spraying operations have been carried out in these gardens since September 2008. Sampling was done weekly from late April to mid-November. It should be noted that no sampling was done from the margins of the gardens. At each sampling time, 80 leaves from all four geographical directions of trees at the height of 1 to 1.8 meters above the ground were randomly selected, and the number of larvae, pupae, and adult insects of C. montrouzieri was counted per leaf. In addition, these leaves were numbered and transferred to the laboratory with the date of sampling in plastic bags. The leaves were examined in the laboratory under a stereomicroscope, and the numbers of each biological stage of the soft scale, as well as the ladybird eggs, were counted and recorded. The data was used to draw the graphs of pest and predator population changes in Excel software.

An analysis of linear regression was done between prey and predator densities in SPSS software to evaluate the type of interaction between *P. aurantii* and *C. montrouzieri*. If P- value > 0.05 or b = 0, the predation would be densityindependent, but if P-value< 0.05 and b > 0 or b< 0, the predator could act as density-dependent and inverse densitydependent, respectively (Kidd and Jervis, 1996).

3. Results

The population fluctuations of the orange pulvinaria scale and its predatory ladybird, *C. montrouzieri*, in 2011 and 2012 are shown in Figures 1-5. The population of soft

scale ovisacs was observed in the first generation from mid-May to late June and in the second generation from mid-August to mid-October (Figure 1). The mean population of scale ovisacs in the first and second generation peaked on June 8 (1.88 ovisacs per leaf) and September 7 (1.8 ovisacs per leaf) in 2011 and also on May 31 (2.04 ovisacs per leaf) and August 30 (1.59 ovisacs per leaf) in 2012 (Figure 1).

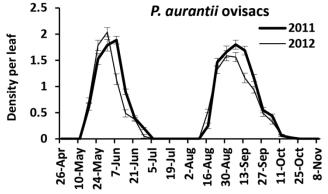


Figure 1. Fluctuations in ovisac population of P. aurantii in citrus orchards of Tonekabon region in 2011 and 2012.

The population of 1^{st} instar nymphs of *P. aurantii* was observed in the first generation from late May to late July and in the second generation from the third decade of August to late October (Figure 2). The mean population of 1^{st} instar nymphs of this pest in the first and second

generations peaked on June 29 (28.65 nymphs per leaf) and September 14 (27.81 nymphs per leaf) 2011, and on June 21 (29.11 nymphs per leaf) and September 6 (21.00 nymphs per leaf) 2012 (Figure 2).

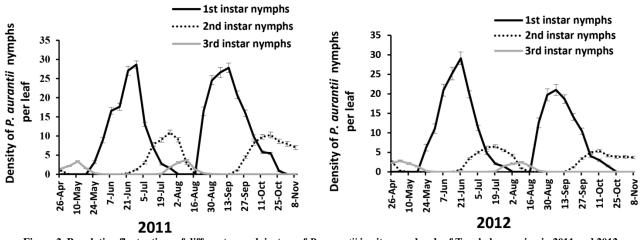


Figure 2. Population fluctuations of different nymph instars of *P. aurantii* in citrus orchards of Tonekabon region in 2011 and 2012.

The population of 2^{nd} instar nymphs of *P. aurantii* was present in the first generation from the third decade of June to mid-August (Figure 2). The emergence of the 2^{nd} instar nymph in the second generation began on September 20, and overwintering of the pest also passed into the 2^{nd} instar nymph. In addition, a small population density was present from early spring to late April. The mean population of 2^{nd} instar nymphs of *P. aurantii* in the first and second generations peaked on 2011, July 27 (10.95 nymphs per leaf) and October 19 (10.27 nymphs per leaf) and October 11 (5.4 nymphs per leaf) (Figure 2).

The population of 3^{rd} instar nymphs from the winter generation of *P. aurantii* was observed from spring to late May (Figure 2). The 3^{rd} instar nymphs of this pest were also present in nature from mid-July to late August. Adult females of *P. aurantii* were also found on plants from early May to June 22, and from early August to mid-September (Figure 3). In 2011, the peak population of 3^{rd} instar nymphs was on May 11 (3.36 nymphs per leaf) and August 10 (3.71 nymphs per leaf), and the peak population of adult female insects was on June 1 (1.8 nymphs per leaf) and August 24 (1.88 nymphs per leaf) (Figure 2). In 2012, the peak population of 3^{rd} instar nymphs was on May 3 (2.79 nymphs per leaf) and August 9 (2.45 nymphs per leaf), and the peak population of adult female insects was observed on May 24 (2.00 adult

insects per leaf) and August 16 (1.53 adult insects per leaf) (Figure 3).

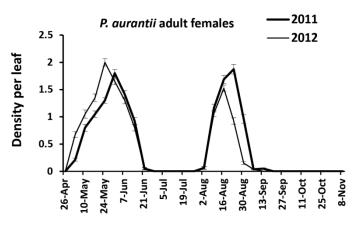


Figure 3. Population fluctuations of P. aurantii adult females in citrus orchards of Tonekabon region in 2011 and 2012.

In both years, the activity of ladybirds began in citrus orchards in May (Figure 4). In 2011, the peak population of *C. montrouzieri* eggs in the first and second generation

of the orange pulvinaria scale was on June 15 (0.14 eggs per leaf) and September 7 (0.19 eggs per leaf), respectively (Figure 4).

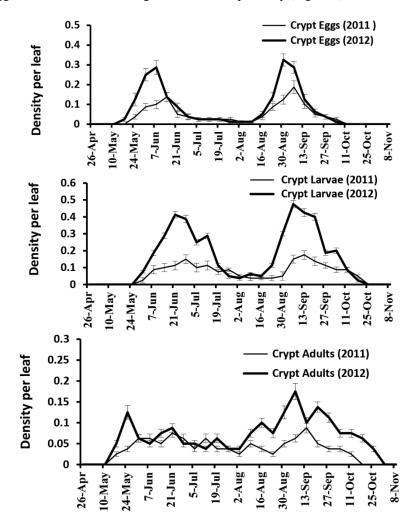


Figure 4. Population fluctuations of eggs, larvae and adult insects of *C. montrouzieri* in citrus orchards of Tonekabon region in 2011 and 2012. Crypt; *C. montrouzieri*.

The peak population of ladybird larvae in two generations of the pest was on June 29 (0.15 larvae per leaf) and September 14 (0.18 larvae per leaf), respectively (Figure 4). In 2012, the peak population of *C. montrouzieri* eggs was in the first and second generation of the soft scale on June 7 (0.29 eggs per leaf) and August 30 (0.33 eggs per leaf), respectively. The peak population of larvae of this ladybird during two generations of pests was on June 21 (0.41 larvae per leaf) and September 6 (0.47 larvae per leaf). The population density of *C. montrouzieri* also ranged from 0.03 to 0.09 per leaf in 2011 and 0.03 to 0.18 per leaf in 2012.

In general, in 2011, the peak of the average population of all biological stages of *P. aurantii* (ovisac, nymph instars, and adult female insects) was on June 29 (30.05 per leaf) and September 14 (29.55 per leaf), and the peak of the average population of all biological stages of *C. montrouzieri* was on June 29 (0.34 per leaf) and also on September 7 and 14 (0.45 per leaf) (Figure 5). In 2012, the peak of the average population of all biological stages of this soft scale was on June 21 (30.09 per leaf) and also on September 6 (22.6 per leaf), and the peak of the average population of all biological stages of predator ladybird was on June 21 (0.65 per leaf) and September 6 (1.00 per leaf).

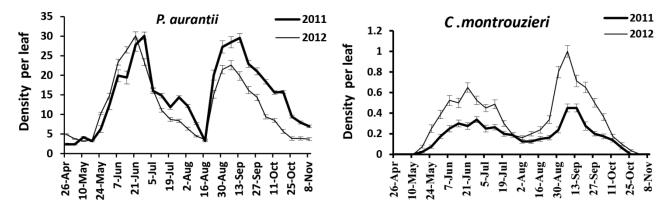


Figure 5. Population fluctuations of P. aurantii and C. montrouzieri in citrus orchards of Tonekabon region in 2011 and 2012.

Regression between population densities of *P. aurantii* ovisacs and *C. montrouzieri* eggs was significant (P-value < 0.001; Table 1), indicating that increasing the prey ovisacs increased the population density of predator eggs.

Further, the P-value of the regression between prey and predator populations was less than 0.01 (b > 0; Table 1), showing a density-dependent reaction of the predator to the prey population (Figure 6).

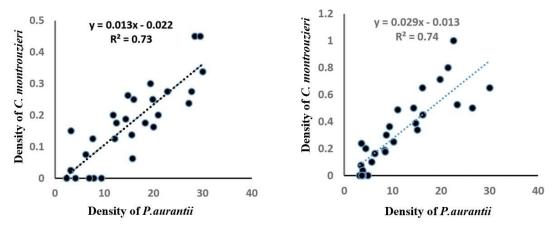


Figure 6. Regression showing the relationship between mean population densities of *P. aurantii* and *C. montrouzieri* in 2011 (left) and 2012 (right). Table 1. Statistics of the linear regression between the mean population densities of *P. aurantii* and *C. montrouzieri* in 2011 and 2012

X-Y ^a		а	b	r	\mathbf{r}^2	Pvalue
P. aurantii Ovisacs– C. montrouzieri Eggs	2011	0.01	0.06	0.82	0.68	0.001 ^b
	2012	0.01	0.13	0.85	0.72	0.001 ^b
P. aurantii – C. montrouzieri	2011	-0.02	0.01	0.85	0.73	0.001 ^b
	2012	-0.01	0.03	0.86	0.74	0.001 ^b

^a Y and X are dependent and independent parameters, respectively. ^b The regression between X and Y is significant at 0.01 level.

4. Discussion

In the process of population changes of the orange pulvinaria scale on the blood orange in the Tonekabon

region, two distinct peaks were observed, which indicate the existence of two generations of this pest per year. This result is consistent with the results of Hallaji Sani (1999), Rajabpour (2006), and Damavandian et al. (2014) that this pest has two generations. The first generation of P. aurantii was observed from May to September, and the second generation of this pest was found from late August to the following June. The present study showed that P. aurantii has a high density in June and September. Also, the peak points of the pest population occur due to the sudden increase in population following the emergence of 1st instar nymphs. In the present study, during 2011 and 2012, different biological stages of P. aurantii were present in nature at almost the same time, but the peak points of this pest were observed in 2011 with a one-week delay compared to 2012, which can be related to changes in weather conditions in these two years. According to meteorological reports, in 2011, there were more rainy days and a decrease in temperature compared to 2012. Abdel-Moniem (2003) showed that the population density of Pulvinaria tenuivalvata (Newstead) was positively related to temperature and relative humidity. In addition to climatic conditions, orange pulvinaria scale activity and population growth can be very closely related to its contamination rate in the previous year.

According to the results of this study, in 2011, there was no significant difference in *P. aurantii* population density between the two generations, but in 2012 this difference was more evident, and the pest population in the second generation decreased considerably. This could be associated with an increase in the population density of the *C. montrouzieri* as an important contributor to changes in the soft scale population (Moore, 1988). The curves of *P. aurantii* and *C. montrouzieri* population changes in this study show that increasing the population of ovisacs, 1st and 2nd instar nymphs of pests, increased predator ladybird populations on infected trees. It can result that these biological stages of *P. aurantii* are favorable for *C. Montrouzieri*.

In this study, the population growth of predator ladybirds in 2012 was faster than in 2011 due to the higher temperature in May 2012 (19 ° C) compared to 2011 (16 ° C) and also the decrease in the number of rainy days in 2012. Hennekam et al. (1987) reported that Cryptolaemus ladybird was unable to be active at temperatures below 17 °C. Besides, it has been reported that the population of Cryptolaemus ladybird on rainy days is lower due to a decrease in suitable prey (Rao et al., 1971). In the experimental gardens, it should be noted that the orange pulvinaria scale was the predominant pest of blood orange trees, and other pests were rarely observed. Thus, the population changes of C. montrouzieri are largely related to the population changes of P. aurantii. In this research, the regression between prey and predator densities (Figure 6) was significant (Table 1), suggesting density-dependent predation. On the other hand, this ladybird feeds more on soft scale eggs than nymphs (Gharizadeh Gelsefidi et al., 2004), hence the cooccurrence of predator ladybird populations with scale ovisacs will play an important role in controlling this pest. Our results showed that the regression between densities of prey ovisacs and predator eggs was significant, indicating the effect of the presence of soft-scale ovisacs

on the increase in the number of eggs laid by predators (Table 1). Further, in the second generation of this pest, more synchronicity was observed between the population of scale ovisacs and ladybird eggs (b > 0). Thus, the peak density of C. montrouzieri eggs coincided with the peak density of citrus P. aurantii ovisacs. Also, the peak of the average population of total biological stages of this ladybird in the first and second generations was observed three weeks and one week later than the peak population of scale ovisacs, respectively. Therefore, in the first generation, when the population of this predator increased, there were not enough P. aurantii ovisacs in which the ladybird could lay their eggs. Consequently, the population density and growth of this predator in this generation were lower compared to the second generation. As a result, C. montrouzieri in 2011 prevented the increase in the P. aurantii population in the second generation. In 2012, due to higher population density, this predator was able to significantly reduce the pest population in the second generation.

In this study, the population density of C. montrouzieri decreased in August in both 2011 and 2012. Solangi et al. (2012) reported that as the temperature rises above 33 °C, reproduction, oviposition, and, consequently, the population of Cryptolaemus ladybird decrease. In our study, every two years, the average temperature in August was 27-28 °C. Therefore, the reduction in the population of C. montrouzieri this month can probably be attributed to the decrease in the favorable stage density of P. aurantii for the predatory ladybird. In September, with the increase of scale ovisacs, the ladybird population also studied increased. Ullah (1992)the population fluctuations of Pulvinaria psidii Maskell and Pulvinaria floccifera Westwood in Bangladesh and mentioned that abiotic factors such as temperature, relative humidity, and rainfall were the main causes of their population changes. Aghajanzadeh and Taheri (2017) showed that the pathogenic fungus Lecanicillium muscarium has an important role in the population reduction of *P. aurantii* in citrus orchards. In a study of population changes of P. pyriformis on the laurel in Greece by Stathas et al. (2009), it was reported that other natural enemies of the pest, such as Chilocorus bipustulatus L. and the parasitoid wasp Metaphycus helvolus (Compere), could not significantly reduce the pest population.

The results of the present study showed that the *C*. *montrouzieri* ladybird has been able to increase its population well over time and settle in citrus orchards, so it plays an important role in *P. aurantii* population changes. In our study, as mentioned before, the ladybird population in the first generation of the pest was less than in the second generation in both years. According to Hagen (1962), the *Cryptolaemus* ladybird does not tolerate temperatures below 10 °C, and a very large population of them perishes in cold winters. On the Black Sea coast, relatively large heat cages are placed around trees for ladybirds, which increase predator survival compared to laboratory breeding and reduce spring release rates (Hodek, 1967). In Azerbaijan, researchers managed to prevent soft-scale outbreaks by releasing 5,000 *C*.

montrouzieri into three hectares of orchards (Prokopenko et al., 1982). Therefore, it is recommended that when we face cold winters in the previous year, the release of this ladybird in citrus orchards should be done at the right time to strengthen its population in order to control this pest effectively.

Acknowledgments

The Research Council of Mohaghegh Ardabili University (Iran) is gratefully acknowledged for their financial support of this research. Also, we are grateful to the Agricultural and Natural Resources Research Center of Mazandaran (Iran) for supporting this study.

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