Article

Determination of economic injury level for first and second generations of *Pulvinaria aurantii* (Hem: Coccidae) in Thomson navel orange orchards

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Abstract

The citrus soft scale, *Pulvinaria aurantii* (Hem: Coccidae) is among the most important pests of citrus orchards in Asia. Damage occurs not only by direct feeding on plant sap, but also by excretion of abundant honeydew which underlies the growth of sooty molds on fruits, leaves, and young twigs. Although, chemical insecticides and mineral oils have long been used by growers to control *P. aurantii*, our current knowledge about the damage and economic injury level of this pest is insufficient. In this study, the economic injury level (EIL) of the first and second generations of *P. aurantii* on Thomson novel orange was investigated during two consecutive years (2011 and 2012). The study unit include a citrus orchard (3000 m^2) located at Babolsar city, north Iran. Four branches of each selected tree with a proximate length of 25 cm were artificially infected by different numbers of *P. aurantii* egg sacs and monitored biweekly to record the number of infected leaves and fruits to both sooty molds *P. aurantii* instars. Finally, the number of fruits infected with sooty molds (more than 50% of the fruit surface) was used to estimate EIL using Pedigo formula. The EIL was calculated as 135, 102, and 125 egg sacs per branches with an average number of 8, 7, and 7 fruits for the first generation of 2011, and the first and second generations of 2012, respectively. These findings may be easily used by local growers to set their control programs based on the density of pest egg sacs on plant surfaces.

Keywords *Pulvinaria aurantii*; economic injury level; mineral oil; damage, egg sac; citrus fruits.

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1 Introduction

The orange soft scale, *Pulvinaria aurantii* (Hem: Coccidae), is one of the most important pests of citrus plants in Asia including Iran, Russia, and Japan (Ebeling, 1951). In addition to citrus plants, this pest may also attack a variety of other hosts, such as *Pittosporum tabira* (bushes), date-plum, mistletoe and rarely loquat and oleander (Davatch and Taghizadeh, 1954; Farahbakhsh, 1970). Damage occurs not only by direct feeding of both nymphs and adult females on plant sap, but also by excretion of high amounts of honeydew, which underlies the growth of sooty molds, and transmission of some plant pathogenic viruses. In high populations, the young fruits and twigs are densely covered by different developmental stages of the pest. The trees infected by high populations of this pest experience inefficient photosynthesis, reduced vigor and loss of leaves and fruits (Behdad, 2003).

During the last decades, the use of chemical insecticides, particularly dursban, diazinon, and buperofosin, has been the main strategy to control *P. aurantii* in Iran (Damavandian, 2007). However, pesticide wash-off by continuous rainfalls, like what is seen in northern Iran, may dilute their effective doses on plant surfaces, thus reduce the efficiency of chemical control. Additionally, the detrimental effects of synthetic pesticides on human, environment and non-target organisms such as natural enemies as well as further problems such as pest resurgence and resistance development by pests has necessitated the exploration of alternative control strategies in the shade of integrated pest management (IPM) concept. The IPM concept proposes the use of a combination of control strategies, such as biological and cultural control, use of semiochemicals and application of more environmentally friendly insecticides with more specificity against the target pest. Additionally, a fundamental concept of IPM is that pest managers should not afford any type of control strategies without considering if this action is economically sound (Alston, 2011). In this context, the usefulness of management actions is determined based on a critical point, commonly known as the economic injury level (EIL). By definition, EIL is the point in which the costs associated with pest management equal the benefits from the pest management actions. Below the EIL there is no need to take pest control actions because they are not economically justified, but economic damages may occur when the pest population exceeds the EIL (Whalon and Craft, 1984; Pedigo et al., 1986).

A large volume of studies has been carried out during the last two decades to determine the EIL for a wide variety of insect pests (Mahmoud, 2014). Artificial inoculation of uninfected host plants and evaluation of the resultant damage has been the most commonly used method to determine EIL (Pedigo et al., 1986). For example, this method has been used to estimate the EIL of the flower thrips, *Frankliniella occidentalis* feeding on red pepper (Park et al., 2007) and sweet pepper (Shipp et al., 1997) and also that of the cotton aphid, *Aphis gossypii* on cotton plants (Afshari, 2007). Despite the greater attention received by greenhouse and field crops, fruit trees have been exposed to a relatively less studies because of difficulties related to estimation of the EIL. In Iran, for example, the EIL of the pistachio psylla, *Agonoscena pistaciae* (Hem: Psyllidae) has been studied by Hasani (2009). Given the sever damages occurred annually by different species of scale insects (superfamily Coccoidea) in citrus orchards of Iran and the lack of proper knowledge on economic injury level of these pests, this study was conducted to estimate the EIL of both first and second generations of *P. aurantii* on a Thomson novel orange orchard located at north of Iran. Healthy fruits were artificially infected by egg sacs of the pest and the relationship between population density and damage was evaluated using routine models.

2 Material and Methods

2.1 Study orchards

This study was carried out during two consecutive growing seasons (2011-2012) to estimate the economic injury level (EIL) of the first and second generations of *P. aurantii*. The study unit includes a orange orchard with an area of 3000 m^3 and 90 trees. The orchard was located at countryside of Babolsar city (Mazandaran province, north of Iran) (52º39' N 36º42' E, 7 meter ASL). All trees were 25-years old Thomson novel orange variety of *Citrus sinensis* on *Citrus aurantium* stand with 5 and 6 m distances between trees and rows, respectively. Chemical pesticides had not ever been used in the sampling area since 2003 and the owner had predominantly relied on natural enemies, and rarely mineral oils, for pest control. Damavandian (2006) showed that 1.03% dose of mineral oil was suitable alternative for pesticide because of its satisfactory effects on first instars of *P. aurantii*. Dividson *et al*. (1991) showed that many key pests of citrus like mites and scales could be controlled with mineral oils. All trees were labeled by an aluminum table covered with plastic to prevent being cleaned by the rain. This study was carried out as a completely randomized design with 6 treatments and 8 replicates (totally 48 trees).

2.2 Damage assessment

In this study, each tree with four branches of about 25 centimeter in length, which contains almost one fruit, was signed as a single replicate. During May 2011 and 2012, the egg sacs containing living eggs of *P. aurantii*, collected from infected citrus orchards, were used to infest the signed branches. The egg sacs were attached on both upper and lower surfaces of leaves. The attachment of the sacs on leaf surface should set out correctly to facilitate the movement and establishment of the emerged crawler on the leaf. The trees were infected by 4, 16, 28, 40, and 52 egg sacs (i.e. 1, 4, 7, 10, 13 egg sacs for each of the four signed branches, respectively) in 2011 experiment. In 2012 experiment, however, a total of 4, 20, 40, 60 and 80 egg sacs (i.e. 1, 5, 10, 15, and 20 egg sacs for each branch, respectively) was inoculated to each orange tree. Uninfected trees were considered as control.

A distance of 50 cm below each experimental branch was pruned in order to monitor and remove other honeydew producing pests such as the Australian giant scale, *Iceria purchasi* and the whitefly, *Bemisia tabaci*. After egg hatching and establishment of the first instars (crawlers), sampling was carried out biweekly and the number of leaves and fruits infested by sooty molds on leaves and fruits were recorded. Dead scales were easily recognizable from live ones using their brown color.

2.3 Estimation of the economic injury level

After collecting required data for both first and second generations of *P. aurantii*, the economic injury level (EIL) was calculated using Pedigo et al. (1986) formula as follow:

$$EIL=P=\frac{C}{V\times I\times D\times K}$$

where C is costs of control, V is costs of yield (fruit) unit (kg), K is the protected product using natural control, I is the damage per unit, and D is the loss per unit. The costs of control were estimated by calculating the costs associated with mineral oils, sprayers and workers. These data were obtained from plant protection operation and inquires farmers about quality of oil spraying with different pumps. The price of yield unit (1 kg Thomson novel orange) was taken from the agriculture ministry pricing in 2011 and 2012. The protected product resulted from natural control (K) is 1 when the pest is controlled completely. According to previous studies, mineral oils (1.03%) cause 90% mortality in pest population, in vitro conditions (Damavandian, 2007), therefore in this study K was considered as 0.9, meaning that 90% of *P. aurantii* damage has been controlled by used mineral oils. it is not possible to separate I and D values because of special type of mouthpart and the observed damages occurred by *P. aurantii* and other scale insects. Since, "I × D" is equivalent to the "b" index, i.e. the slope of the regression line in y=a+bx formula (Pedigo et al., 1986), so the equation and regression relation between the number of egg sacs in the four branches and the observed damages on fruits were estimated and considered as I × D. In this study, the economic injury level (EIL) was calculated according to infested fruits to sooty molds as the main cause of reduced marketability of citrus plants in Iran.

The regression equation between infested leaves to *P. aurantii* at the beginning of season and damaged fruits (i.e. fruits infected with sooty molds for more than 50% of their surfaces) were calculated. To determine the regression equation type between different densities of egg sacs and damaged products as well as the infested leaves to the pest and the fruits infested by sooty molds, the fitting reversal test of SPSS software was used to clarify if these regression equations were linear or not. The equation with the most correlation coefficient was finally selected for estimation of the economic injury level (EIL). Furthermore, the average number of infested fruits, blackened leaves, infested leaves to the pest and controlled leaves with entomopathogenic fungi in different treatment were analyzed Using Tukey test of SPSS software at $P<0.05$.

3 Results

3.1 Damage assessment

The soft scales were observed to establish on leaves and fruits 1-2 weeks after attaching egg sacs on selected branches. Significant proportion of the pest was observed to establish on the lower surface of leaves, especially along the main longitudinal vein. Nearly all individuals established on upper surface of leaves were eliminated probably due to susceptibility to harsh environmental factors such as sunlight and washing off by the rain. After residing on plant surfaces, the first instar larvae (crawler) lose their locomotory organs and start to feed on plant soap and produce the honeydew that attract the black sooty mold on fruits and leaves. Those fruits with at least 50% contamination to the black sooty molds are less marketable, thus were grouped as damaged fruits in this study. The uninfected fruits or those with less than 50% contamination to black molds were considered as healthy fruits.

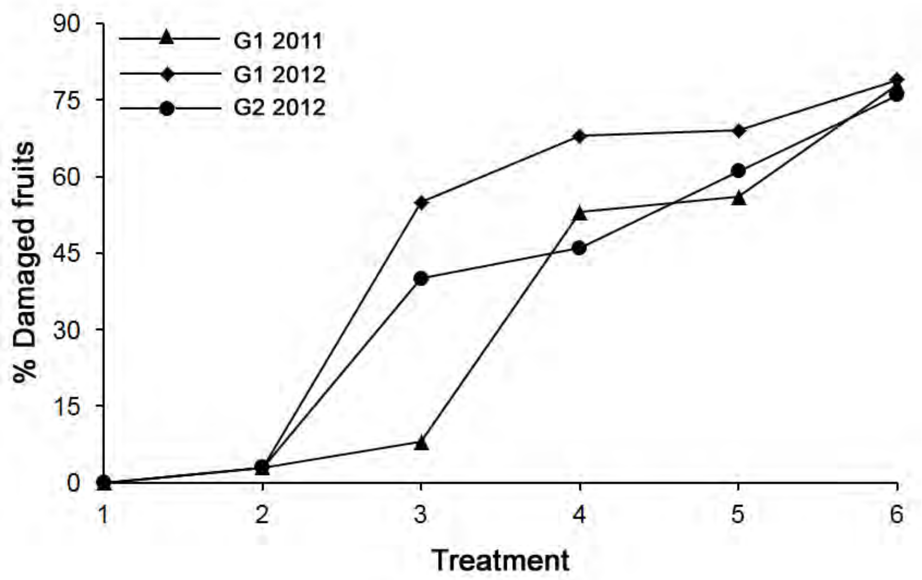

Fig. 1 Damage of *Pulvinaria aurantii* on citrus fruits infected by different egg sac densities in two consecutive years, Treatments 1-6 included control, 4, 16, 28, 40, and 52 egg sacs per tree in 2011, and included control, 4, 20, 40, 60 and 80 egg sacs per tree in 2012.

3.2 Damage quantification

The average percentage of damaged fruits in first generation of 2011 study and first and second generations of 2012 study have been shown in Fig. 1. An ascending pattern in the number of damaged fruits was observed by increase in the number of egg sacs deposited on plant surfaces with the most damages (about 70%) were observed in trees infected by the highest number of egg sacs in all generations (Fig. 1). Although, the branches were infected by lower numbers of egg sacs at the first generation of 2011 (1, 4, 7, 10, and 13 egg sacs per branch) compared to 2012 (1, 5, 10, 15, and 20 egg sacs per branch), the observed damage was not statistically different from the damages occurred in both first and second generations of 2012 (Fig. 1). These differences were even less evident when the branches were infected by higher numbers of egg sacs. This may imply that the intensity of damage decreases in high densities of pest population probably due to increased competition and mortality as well as attracting higher numbers of natural enemies.

The average numbers of infected and blackened leaves and the average number of blackened fruits in trees infected by different densities of *P. aurantii* egg sacs in 2011 have been summarized in Table 1. The average numbers of infected and blackened leaves in low density of the pest were not statistically different from control. In higher egg densities, however, significant increases in these values were observed. Similarly, the average numbers of blackened fruits in trees infected by 4 and 16 egg sacs were not statistically different from control, but the number of damaged fruits increased as a result of increased number of released egg sacs to 28, 40 and 52 egg sacs per tree (Table 1).

Table 1 The average numbers of leaves and fruits infected by instars of *Pulvinaria aurantii* and sooty molds in the first generation of 2011, different letters show significant differences at 0.05 level, comparisons have been made only within columns (ANOVA).

Treatment	No. egg sacs per tree	No. leaves infested to *P.aurantii*	No. blackened leaves	No. blackened fruits
1	0	0^{a}	0^{a}	0^{a}
2	4	7^{a}	0.93^{a}	0.125^{a}
3	16	24.5^{b}	3.84^{ab}	1^{a}
4	28	34.62^{b}	7.25^{bc}	2.125^{b}
5	40	47.75^{c}	10.37^{cd}	2.25^{b}
6	52	58.37^{c}	15.5^{d}	3.125^{b}

The average numbers of infected leaves, blackened leaves and blackened fruits in trees infected by different densities of *P. aurantii* egg sacs in early 2012 (first generation) have been summarized in Table 2. Like the first generation of 2011 experiment, we found no significant difference in all mentioned values among control and trees infected by the lowest density of egg sacs (4 egg sacs per tree). However, the average number of blackened leaves and fruits increased significantly when the experimental trees were infected by higher densities of egg sacs (Table 2). Increase in the number of egg sacs from 60 t0 80 led to significant increase in the number of infected and blackened leaves, but not the number of blackened fruits (Table 2). This may be explained by the low number of fruits existing on each experimental tree. A nearly similar pattern of increase in the number of damaged leaves and fruits were observed in the second generation of the pest (Table 3). Like the first generation, we found significant differences between the number of infected and blackened leaves, but not blackened fruits, when the number of egg sacs increased from 60 t0 80 sacs per tree (Table 3).

Table 2 The average numbers of leaves and fruits infected by instars of *Pulvinaria aurantii* and sooty molds in the first generation of 2012, different letters show significant differences at 0.05 level, comparisons have been made only within columns (ANOVA).

No. blackened fruits	No. blackened leaves	No. leaves infested to *P.aurantii*	No. egg sacs per tree	Treatment	No. egg sacs at the end of generation
1	0	0^{a}	0^{a}	0^{a}	0^{a}
2	4	7^{a}	0.93^{a}	0.12^{a}	1.5^{ab}
3	20	24.12^{b}	10.84^{b}	3^{b}	6.5^{bc}
4	40	30.37^{b}	13.85^{b}	4^{bc}	8.25^{cd}
5	60	41.12^{c}	17.5^{b}	4.37^{c}	13.37^{de}
6	80	51.5^{d}	29^{c}	5^{c}	16.12^{e}

Table 3 The average numbers of leaves and fruits infected by instars of *Pulvinaria aurantii* and sooty molds in the second generation of 2012, different letters show significant differences at 0.05 level, comparisons have been made only within columns (ANOVA).

Treatment	No. egg sacs per tree	No. leaves infested to *P.aurantii*	No. blackened leaves	No. blackened fruits
1	0	0[a]	0[a]	0[a]
2	4	4.62[a]	0.93[a]	0.125[a]
3	20	19.5[b]	3.37[a]	2.25[b]
4	40	25.37[bc]	9.62[b]	2.25[b]
5	60	30.27[c]	12.5[b]	3.37[bc]
6	80	39.62[d]	16.37[c]	4[c]

3.3 Calculation of economic injury level

The cost of control (C index in EIL formula) was estimated by summing the cost of consumed mineral oil per tree and the rent cost of sprayer and worker per tree. The cost of consumed mineral oil (1/3 liter, 330 CC) was estimated 6500 and 1000 Rial for the growin seasons of 2011 and 2012, respectively. The rent costs of sprayer and workers per tree were also calculated as 6200 and 6700 Rial for 2011 and 2012, respectively. Therefore, the overall cost of *P. aurantii* control for each citrus tree was estimated to be 12700 Rial and 16700 Rial for 2011 and 2012, respectively.

As previously was mentioned, the slop of regression line (b) between the number of released egg sacs (x) and the number of blackened fruits by sooty molds (y) is usually considered as the value of I×D in EIL formula. The results of fitting reversal test of SPSS that shows linear or nonlinear regression models, showed that the equation in our study system were linear for all three studied generations. The constructed regression equations were estimated as y=0.06x+0.019 (R^2=0.738) for first generation in 2011 experiment, and y=0.073x+0.53 (R^2=0.739) and y=0.049x+0.32 (R^2=0.723) for first and second generations in 2012 experiment, respectively (Fig. 2).

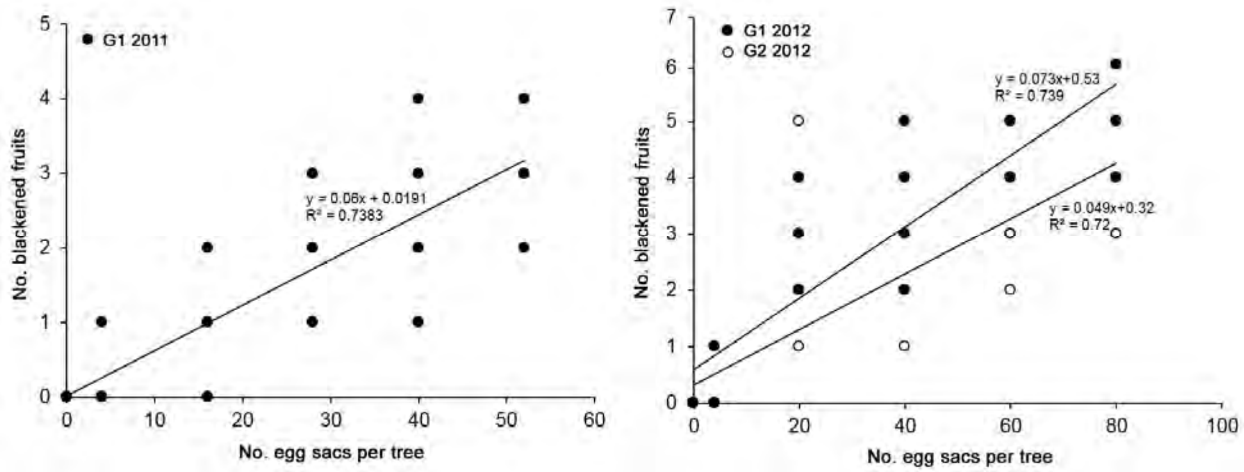

Fig. 2 Regression relationships between the number of *Pulvinaria aurantii* egg sacs and the number of blackened fruits for first generation of the pest in 2011 and for first and second generations in 2012.

The price of product unit (1 kg Thomson novel orange) was obtained from the government pricing as 7000 and 10000 Rial for 2011 and 2012, respectively. In this study, the infection of fruits to sooty molds was considered as the main damage for calculation of EIL. To obtain the price for each individual fruit, at the end of the experiments, all fruits were picked up and their weights were measured. Averagely, each four fruits were found to weigh one kilogram, so the price of individual fruits was estimated to be about 1750 and 2500 Rials in 2011 and 2012, respectively. Given that the successful management coefficient (K) of the pest using mineral oils was 0.9, the economic injury level for the first generation of 2011 and the first and second generations of 2012 were calculated as follows:

First generation in 2011: (EIL= 1270/ (1750 × 0.06× 0.9) = 134.39≃135)

The number of infected fruits in trees infected by 28 egg sacs was not statistically different from those infected by higher numbers of egg sacs (Table 1). Therefore, the presence of about 135 egg sacs on 25 cm length of fruit-bearing branches seems to be the economic injury level. According to the gain threshold, which was calculated 8 fruit for each tree (Gain threshold=C/V= 1270/175=7.25≃ 8), this number of egg sacs should exist on branches with a total number of 8 fruits.

First generation in 2012: (EIL: 1670/ 250× 0.073× 0.9) = 101.67≃ 102)

The total number of infected fruits in trees infected by 20 egg sacs was not statistically different from those infected by 40 egg sacs, although it was different from trees infected by higher numbers of egg sacs (Table 2). Given that 20 egg sacs per tree caused significant (55%) and with respect to the gain threshold calculated as 7 fruits (C/V= 1670/ 250= 6.68≃ 7), the presence of 102 egg sacs on 25 cm length of branches containing a total number of 7 fruits should be considered as economic injury level for the first generation of this pest at 2012.

Second generation in 2012: (EIL: 1670/ (250× 0.049×0.9) = 151.47 ≃152)

Similar to the first generation, as the average number of infected fruits in branches infected by 5, 10, or 15 egg sacs were not statistically different, therefore, the presence of 152 egg sacs in 5 sets on branches should be considered as the economic injury level for the second generation at 2012. By knowing the gain threshold for this year (7), this number of egg sacs should be distributed across branches, which contain a total number of 7 fruits.

4 Discussion

In this study, the economic injury level of the citrus soft scale, *P. aurantii* was investigated for two consecutive growing seasons. *P. aurantii* is among the most important pests of citrus plants in some parts of Asia including Russia, Japan, and Iran. Despite its economic damages on citrus productions in Iran, inaccurate information about the economic injury level of this pest has led to unprincipled use of chemical insecticides and mineral oils in inappropriate doses and times. Besides harmful effects on human health, environment and underground and aboveground waters, these insecticides have caused a significant decrease in abundance of some biological control agents such as the ladybugs, *Cryptolaemus montrouzieri* and *Chilocorous bipustulatus*, the predatory mite acari, *Allothrombium pulvinum*, and the entomopathogenic fungi, *Verticilium lecanii* (Saboori et al., 2003; Damavandian, 2007).

We used the artificial infestation of healthy plants to study the correlation between pest densities and observed damages on fruits, a common method currently used by many researchers for estimation of the economic injury level in different insect species (Wightman et al., 1995; Park et al., 2007). Results of the current study showed that the economic injury level for the first generation of *P. aurantii* in 2012 was lower than that of 2011. This reduction may be related to the increase in economic cost of the product, cost of control and accuracy of the experimental design. The economic injury level in the second generation was estimated to be higher than that of the first generation in 2012. The increased mortality of the first instars in the second generation, probably due to adverse weather and biological control agents, may explain the decreased economic injury level in this generation.

Different damage indices and developmental stages have been used by different researchers to estimate the economic injury level. For example, Jemsi (2007) used the density of pest on surface unit to estimation the economic injury level in the grain leaf miner *Syringopais temperatella* Led. (Lep: Elachistidae), while Bahrami *et al.* (2003) and Naranjo et al. (1996) studied the number of insects per bush for the sunn pest, *Eurygaster integriceps* (Hem: Scutelleridae) and the whitefly, *Bemisia tabaci* (Hem: Aleyrodidae), respectively. Kovanci *et al*. (2005) used the number of trapped insects as an index for EIL estimation. Obviously, none of these mentioned methods is applicable for fruit trees. Rajabpour et al. (2008) divided citrus trees to four distinct groups in term of infection levels based on a variety of factors including the area of tree covered by sooty molds, the area of tree infected by egg sacs and the relative density of egg sacs on trees. These authors estimated that the presence of 5-8 first and second instars of *P. aurantii* on 24 leaves of infected trees as the economic injury level of the second generation of this pest in 2008. As recognition of the first instars was difficult for citrus growers, we used the number of egg sacs on young branches as a new index of EIL estimation to simplify the observation, identification and exact estimation of the economic injury level. This index can easily be used by local growers to estimate the intensity of damages on their fruits and set management programs using their available tools.

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