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The Potential of *Sympherobius pygmaeus* (Rambur, 1842) as a Biological Agent Against *Planococcus citri* (Risso, 1813) in Citrus Orchards

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ABSTRACT

Planococcus citri (Risso) (Hemiptera: Pseudococcidae) is one of the most significant pests for especially citrus, crop plants and ornamental plants. Besides its worldwide importance, chemical control is a significant, major method to suppress the population of *P. citri* in Turkey. Moreover, biological control has become more important in recent years because of the increase in consumer consciousness about pesticides. For this reason, the population dynamic of *P. citri* and its predator, *Sympherobius pygmaeus* (Rambur) (Neuroptera: Hemerobiidae) in three different grapefruit orchards were studied. For the study, 10 trees were randomly chosen from each orchard and the individuals on the plants, 4 branches, and stem of each tree were counted to determine the population level. When *P. citri* population reached the peak level, approximately 500 *S. pygmaues* individuals were released to each orchard. Population levels of prey and predators were monitored for two years, with weekly intervals between May-October and one time for two weeks between December and April of the following year. As the result of the treatment process, it is observed that the predator *S. pygmaeus* succeeded in decreasing the population of *P. citri* under economic damage threshold level. As a result, it is demonstrated that the predator *S. pygmaeus* can be used as a biological agent against *P. citri* in integrated pest management programs as an environmentally friendly method.

Key words: Hemerobiidae, Neuroptera, mealybug, biological control, grapefruit, population.

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INTRODUCTION

Citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae)is an important pest for many fruit trees and ornamental plants, especially for citrus in Turkey (Kansu & Uygun, 1980; Polat, Ulgenturk, & Kaydan, 2008; Uygun & Satar, 2008; Karacaoğlu & Satar, 2017). It feeds on fruits and twigs of citrus by sucking the sap and causes dark-colored sooty mold and fugacity (Karacaoğlu & Satar, 2017). It also opens the way to secondary pests like *Cryptoblabes gnidiella* Millière (Lepidoptera: Pyralidae). *P. citri* prefers Washington navel and Star ruby rather than Interdonata lemon and Satsuma mandarin. On the other hand, it cannot complete a generation on Okitsu Wase mandarin variety (Canbolat, 2016). The grapefruit fruits are in the tree as cluster, so it is a great harbor for mealybug as it is protected from unfavorable weather conditions such as extreme heat or low humidity and from various big predators such as *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) adult. Through this protection, insecticide cannot reach this fruit, and mealybug control can be unsuccessful on the grapefruit. Chemical and biological control are widely used methods for suppressing pests both in our country and around the world. There are various negative effects of chemical control on human health and environment such as residue on fruit, resistance development and having effect on non-target organisms; in order to be able to prevent and control such effects, biological control provides an alternative for farmers. Thirty native predators and parasitoids on *P. citri* in the citrus orchards of East Mediterranean Region, Turkey have been recorded until today. Moreover mealybug control in citrus orchards has been done both with natural enemies such as *C. montrouzieri* and *Leptomastix dactylopii* How. (Hymenoptra: Encyrtidae) which are commercially available for a long time (Soylu & Ürel, 1977; Kansu & Uygun, 1980; Türkyılmaz, 1986; Uygun & Satar, 2008; Yayla & Satar 2012; Karacaoğlu & Satar, 2017). Change in vegetation pattern and pesticide use pattern, especially in broad spectrum pesticide with Chloranicotinyl Insecticide (CNI) group instead of summer oil and cultural practices, have resulted in insufficient biological control tactics in grapefruit orchards because of the biology of mealybug on grapefruit, which is in abetter position for *P. citri* when compared to other citrus varieties (Canbolat & Satar, 2016). At this point, importance of alternative biological control agents increases to support current biological control tactics; in addition, the process may sometimes become a solution. *S. pygmaeus* is a promising natural enemy that controls mealybug. Easy production in laboratory, low labor requirement, and rapid growth puts the predator forward for this project (Yayla & Satar, 2012; 2013). Therefore, the biological control of *P. citri* determined by using *S. pygmaeus* under field conditions. For this aim, *S. pygmaeus* was released to citrus plantations to detect its efficiency on mealybug in field condition; population dynamics of both prey and the predator were carefully observed in the study.

MATERIALS AND METHODS

Production of sprouted potatoes

Potatoes used for the experiment were stored in a dark room at +4 ˚C. Then the potato tubers were washed with tap water and cleaned. The cleaned potato tubers were placed in the climatics room with 20 ± 4 ˚C temperature and at 60 ± 10% relative humidity condition. It was observed that, sprouts began to appear on the tubers in 30 to 45 days. Sprouts reaching about 4 to 5 cm in length were used for mass production of *P. citri*. Each time the process were started from new potatoes which were kept in the +4 ˚C This process continued during the 2014 and 2015.

Planococcus citri rearing

Planococcus citri were reared on sprouted potatoes in cages in a climate controlled room with 25 ± 2 ˚C temperature, at 60 ± 10% relative humidity and were waited for 16: 8 hours (light: dark) daily light period (Yayla & Satar, 2012). *P. citri* were obtained from the citrus orchards of Çukurova University, Faculty of Agriculture, Research and Implementation Farm and transformed into the pure culture by subculturing at the egg stage of P. citri several times on the sprouted potatoes (Fisher, 1963).

Sympherobius pygmaeus rearing

The Neuropteran adults were collected from *P. citri* infected shoots and fruit clusters through a Steiner funnel before rearing from 20 years old grapefruits orchards at Çukurova University, Faculty of Agriculture, Research and Implementation Farm. These individuals were classified according to their morphological characteristics under Leica S8APO binocular microscope in the laboratory, and *S. pygmaeus* individuals belonging to Hemerobiidae family were distinguished by following character combination; anterior radial tarce with at least 2-12 radial sectoral branches and nygmata absent (Oswald, 1993). Moreover as it is name their color commonly brownish or greyish compare to other Neuroptera family (New, 2001). The adults collect from were released on mealybugs which were reared on the sprouted potatoes, in a five liter plastics jars in the insect production rooms with 25 ± 2 ˚C and 16 h day light conditions. Each of the production unit have 5-10 sprouted potatoes and released *S. pygmaeus* adults in the unit finding enough place to continue its life. Before starting rearing process, sponges or fiber-like materials were placed in jars for individuals egg laying. After first generations the obtained adult were sent to Prof. Dr. Norm Penny at California University for identifications. After identification and several generation later the adult individuals were start to use in the experiment.

Population dynamic

Population studies were carried out in three different regions to investigate the activity of *S. pygmaeus* and its prey *P. citri* in the citrus orchards. Alata Horticultural Research Institute in Erdemli district of Mersin province, Subtropical Fruits Research and Application Center in Çukurova University and a farmer orchard in Erzin district of Hatay were selected for this aim and population dynamic of the predator and its prey were observed on randomly selected 10 trees within experimental area. The trees were selected from *S. pygmaeus released area* which have generally 40-50 citrus trees during the 2014-2015. *P. citri* population on four main branches at different directions and 10 fruits on each one of 10 trees for every week in each orchard was observed. Totally, 10 trunks, 40 branches and 100 fruits were examined in each orchard. At each sampling point, egg packs, nymph1+nymph2, nymph3 stages and adults of *P. citri* individuals were counted separately and population fluctuations were determined accordingly. When *P. citri* population reached a peak level, approximately 10 *S. pygmaeus* adults per tree were released into the trees. The released individuals did not caged at all. Larvae stages and *S. pygmaeus* adults were taken into account while determining the population by using Steiner funnel (Steiner, 1962) and/or through naked eyes. Population of prey and predator were recorded once a week from April 2014 to December 2015.

RESULTS AND DISCUSSION

Sympherobius pygmaeus suppressed *P. citri* populations in three grapefruit orchards successfully and showed that it is an effective predator on its prey. *P. citri* individuals that overwinter as adult or at 3rd Nymph stage started to lay egg package actively at end of April 2014 in the grapefruit orchard of Çukurova University Subtropical Fruits Research and Application Centre (Fig. 1). Mealybug reached its first peak on June 19th, 2014, with mean 0.30 egg packs/branches. However, when it reached the first peak, summer oil was applied to control for other citrus pests. As a result of this spraying, a decrease in *P. citri* population was observed. The pest reached to second and highest peak level on August 8th, 2014 as mean 5.18 Nymph 1st + 2nd/fruit and 1.23 Nymph 3rd/fruit. After *S. pygmaeus* individuals were released on the trees following population increase of *P. citri* on July 24th, 2014, mealybug population began to decrease in the next 15 to 20 days. It was observed that the larvae of the predator first fed on the egg packages. Then, seven adult individuals were caught in the field with Steiner funnel on September 11st, 2014. When the data were examined, it was determined that *P. citri* population was suppressed within two months after the releasing of the predator. *P. citri* population data in the first year was in line with the data of the second year.

In the second year, the pest reached the first peak on June 5th 2015 and mean 0.50 egg packages/trunk were counted. Mean 0.38 Nymph 1st + 2nd/fruit were counted on August 14th, 2015 as the second peak. *S. pygmaeus* were released on the 24th of July 2015 due to the increasing mealybug population. Five adults were caught with Steiner funnel on September 18th, 2015. *S. pygmaeus* adults were found in all samples taken from the field surveys for a whole year. For instance, two larvae were found on January and February in 2015. *P. citri* population was suppressed by *S. pygmaeus* individuals within 45 days after the release of *S. pygmaeus* in the second year (Fig. 1). When two years were compared, it was observed that the second year had lower population density except adult on trunk. A turbo atomizer without fan is used for spraying the insecticide in this farm. It couldn't be effective on 40-60 cm above ground, as there is no fan. So the insecticide couldn't reach the trunk and *P. citri* adults used the trunk as reservoir; crawlers could spread out from trunk to the upper parts of trees and keep the population active.

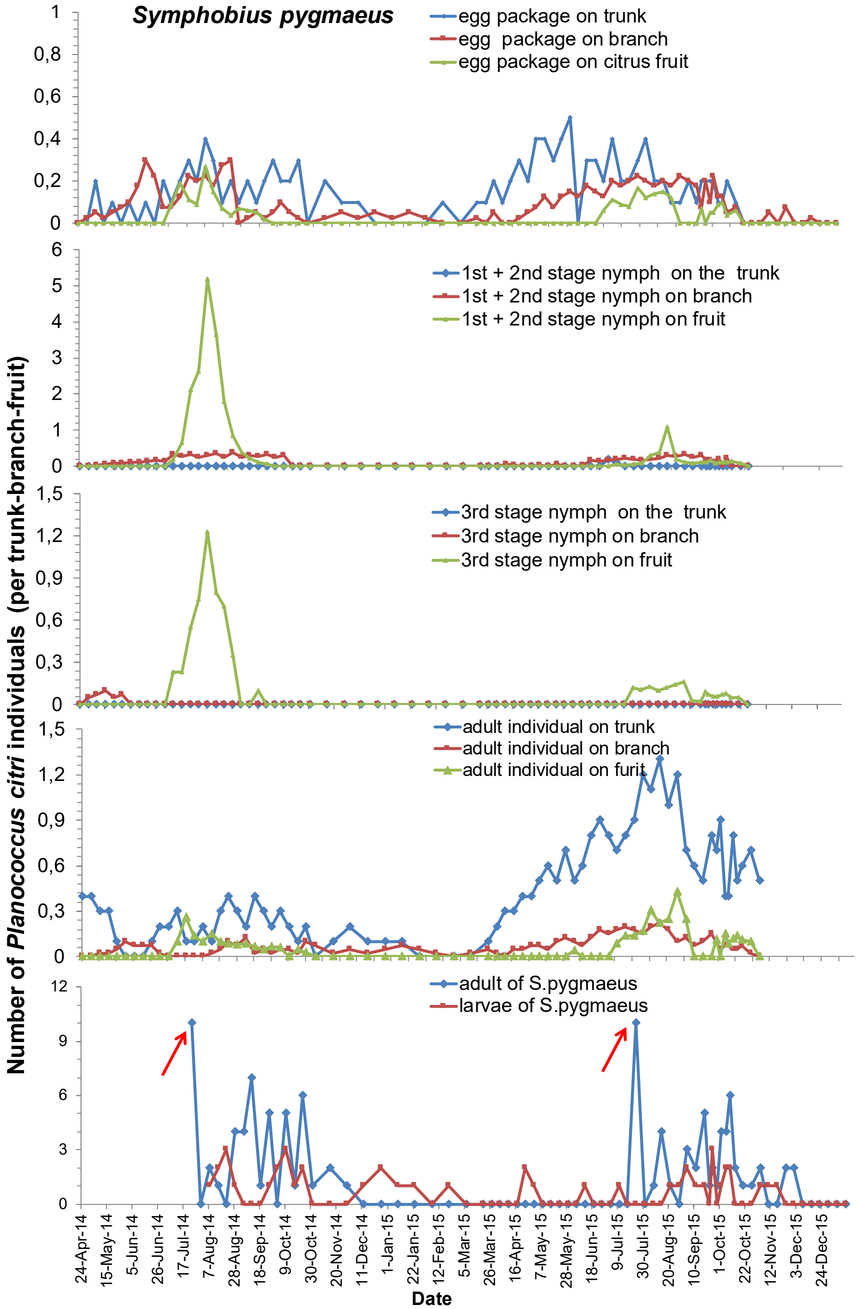

Fig. 1. Population fluctuation of *Sympherobius pygmaeus* and *Planococcus citri* grapefruit orchard in Çukurova University Subtropical Fruit Research and Application Center from April 2014 to December 2015 (arrow shows *S. pygmeous* releasing date).

Planococcus citri activity was observed as egg package, third nymph stage and adult individuals in the first week of May in 2014 at Alata Horticultural Research Institute in the Erdemli district of Mersin province. The mealybug reached its first and highest peak on May 17th, 2014, and were counted as mean 5.9 eggs packages/trunk; 29.7 Nymph 1st + 2nd/trunk, 7.4 Nymph 1st + 2nd/brunch; 6.20 Nymph 3rd/trunk, 1.48 Nymph 3rd/brunch. Second pick is on June 21st, 2014 as 24.5 Nymph 1st + 2nd/fruit, 9.2 Nymph 3rd/fruit, and 4.96 adult/fruit. *S. pygmaeus* individuals were released on June 21st, 2014 after the rapid increase in *P. citri* population and suppressed the pest. As a result of Steiner funnel sampling after the releasing on *P. citri* infected fruits, 54 larvae and 37 adult *S. pygmaeus* individuals were collected (Fig. 2).

Planococcus citri population in the second year was lower than the previous year. As can be seen in Figure 2, the pest reached its first peak on May 23rd, 2015 and mean 1.2 eggs were counted on the trunk. Nymph 1st + 2nd and Nymph 3rd were counted as approximately 1.12 and 0.75 individuals on fruit in June 2015, respectively. Adult individuals on fruits reached the highest level on May 30, 2015 and 1.7 adult individuals were observed on trunk. *P. citri* population began to decrease in 25-30 days following the releasing of *S. pygmaeus* on June 6th, 2015. Eight *S. pygmaeus* individuals were obtained from infected fruit samples with mealybug by using Steiner funnel in July 18th, five individuals were obtained in August 22nd, and eight individuals were obtained in September 10th. It was observed that *S. pygmaeus* individuals were active throughout the year except winter (Fig. 2). Although significantly high population density of the mealybug is observed in the first year, it was suppressed in the short term by *S. pygmaeus. S*econd year *P. citri* population didn't reach a high level as the predator settled in that area

When Figure 3 is examined, it can be seen that the pest reached its first peak in June 15th, 2014 and 3rd nymph stage on the trunk were observed in grapefruit orchard in Erzin. Hatay province. Nymph 1st + 2nd stages were determined to be 5.9 average on the trunk on the 6th of July 2014. 1.2 adult/trunk were counted on June 27th, 2014. Declines began in *P. citri* population in 20-35 days after releasing *S. pygmaues* on the 20th of July, 2014. *S. pygmaues* obtained with Steiner funnel was highest with seven individuals on October 5th, 2014. *P. citri* population was lower than the previous year according to the data in 2015. The first peak on *P. citri* population was 0.3 adult/trunk on May 17th, 2015 and 1.4 Nymph 3rd/trunk on July 5th, 2015. Maximum population level was 9.1 Nymph 1st + 2nd stages/trunk on July 19th, 2015. After releasing of *S. pygmaeus* on the 19th of July, 2015, *P. citri* population began to decrease in 20-30 days. *S. pygmaeus* individuals reached the peak on August 30th, 2015 with 5 individuals. *S. pygmaeus* population was low in both years, as there was an uncontrolled spraying against the disease and pests by farmers and untargeted natural enemies like *S. pygmaeus* were destroyed. Six peaks were detected in each grove in the months of May on main trunk, June, July, August and September on fruit and finally in October-November on fruit trunk during 2014 and 2015. Karacaoğlu & Satar (2017) reported that, there can be 4 generations of the same fruit in the same geographic area, but the number of generations changes according to climatic factors;

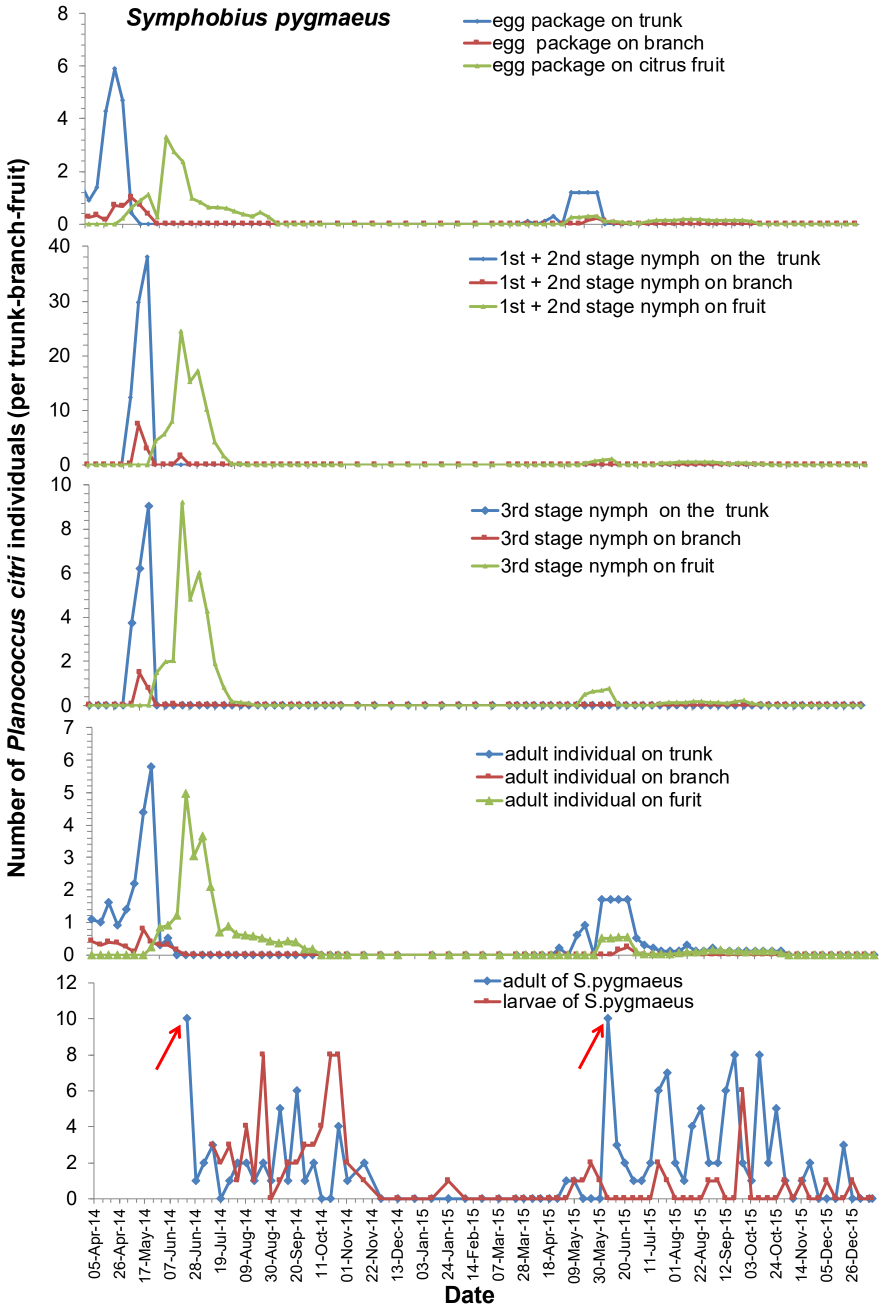

Fig. 2. Population fluctuation of *Sympherobius pygmaeus* and *Planococcus citri* in grapefruit orchard in Alata Horticultural Research Institute in Erdemli district of Mersin province in 2014 and 2015 (arrow shows *S. pygmeous* releasing date).

it is also reported that *P. citri* can give two to three generations in California (Ebeling, 1959); three generations in Texas (Harlan, Hart, Ingle, & Mayerdirk, 1977); eight generations in Palestine (Bodenheimer, 1951); 4-5 generations in Greece (Santorini, 1977); 5 generations in Spain (Martínez-Ferrer, Garcia-Marí, & Moles, 2003). Avidov & Harpaz (1969) reported that citrus mealybug can give six generations per year in coastal plain, 4-5 generations in hill region and up to 7 generation in upper Jordan Valley based on 8.2˚C developmental threshold and 698 days-degree for development of one generation. Karacaoğlu & Satar (2017) calculated 8.53 ˚C developmental threshold and 666.67 days-degree for one generation (from egg to egg stage). *P. citri* can generally hibernate the trunk of citrus tree till the end of April and at this point they give first generation on the trunk, hatched crawler feeding branch and leaf of the tree and moving up and settle under the calix when fruits reach 2-3 cm Ø (Figs. 1, 2 and 3).

In the project, not only *S. pygmaeus* but also other predators, Coleoptera and Neuroptera orders were collected. Seven species and one genus level in Coccinellidae family (Coleoptera), one species from Hemerebiidae, three species from Chrysopidae and Coniopterygidae family (Neuroptera) were identified (Table 1). *Chrysoperla carnea,* which is common predator species, was obtained at highest number followed by *S. pygmaeus.* All *C. carnea* individuals were adults and possibly came to feed on honeydew excreted by mealybug. Only a few individuals could be collected from *Nephus includens* which is a native and a mealybug specific predator. Erzin has the poorest species richness and lower predators than Adana and Alata (Erdemli).

Sympherobius pygmaeus is worldwide available but it is rather neglected by researchers, as the related literature is generally limited with taxonomic studies (Pröse, 1995; Bayram, 2008; Duelli, Bolt, & Henry, 2015). In our country, *S. pygmaues* was firstly detected by (Şengoca, 1979) in Kahramanmaraş province. In addition, Türkyılmaz (1984) reported that it feeds on mealybugs in citrus fruits in Antalya region.

According to the study of Türkyılmaz (1986) in citrus orchards in Antalya province, *S. pygmaues* population's feed on *P*. citri increased in parallel with the increase in mealybug population and reached the highest level in July, which is similar to the findings of this study. Moreover, The researcher revealed that *S. pygmaues* is the most important predator of *P. citri* and also he indicated the population development between prey and predator. However, Bozdoğan, Özcan, Satar, & Tusun (2016) surveyed pasture, wooded, swamp, and heath land areas between 2011 and 2013 in Osmaniye and found that *S. pygmaeous* reached the peak level on May. It can resulted from the fact that the chosen areas were non-cultured.

Martínez-Ferrer et al. (2003) emphasized that *P. citri* population started to increase at end of June through mid of July, but the population decreased when predator and parasitoid occurred. Karacaoğlu (2016) also studied the relationship between *P. citri* population and the predator population in citrus fields in Aegean and Mediterranean regions. The study was carried out in 2013-2015 by using Steiner (Steiner, 1962) and visual control methods in citrus orchards. As a result of this study, 70 adult from *S. pygmaues* species belonging to Hemerobiidae family and 239 adult individuals from *S. fallax* species were identified. Study of Karacaoğlu (2016) revealed a direct relationship

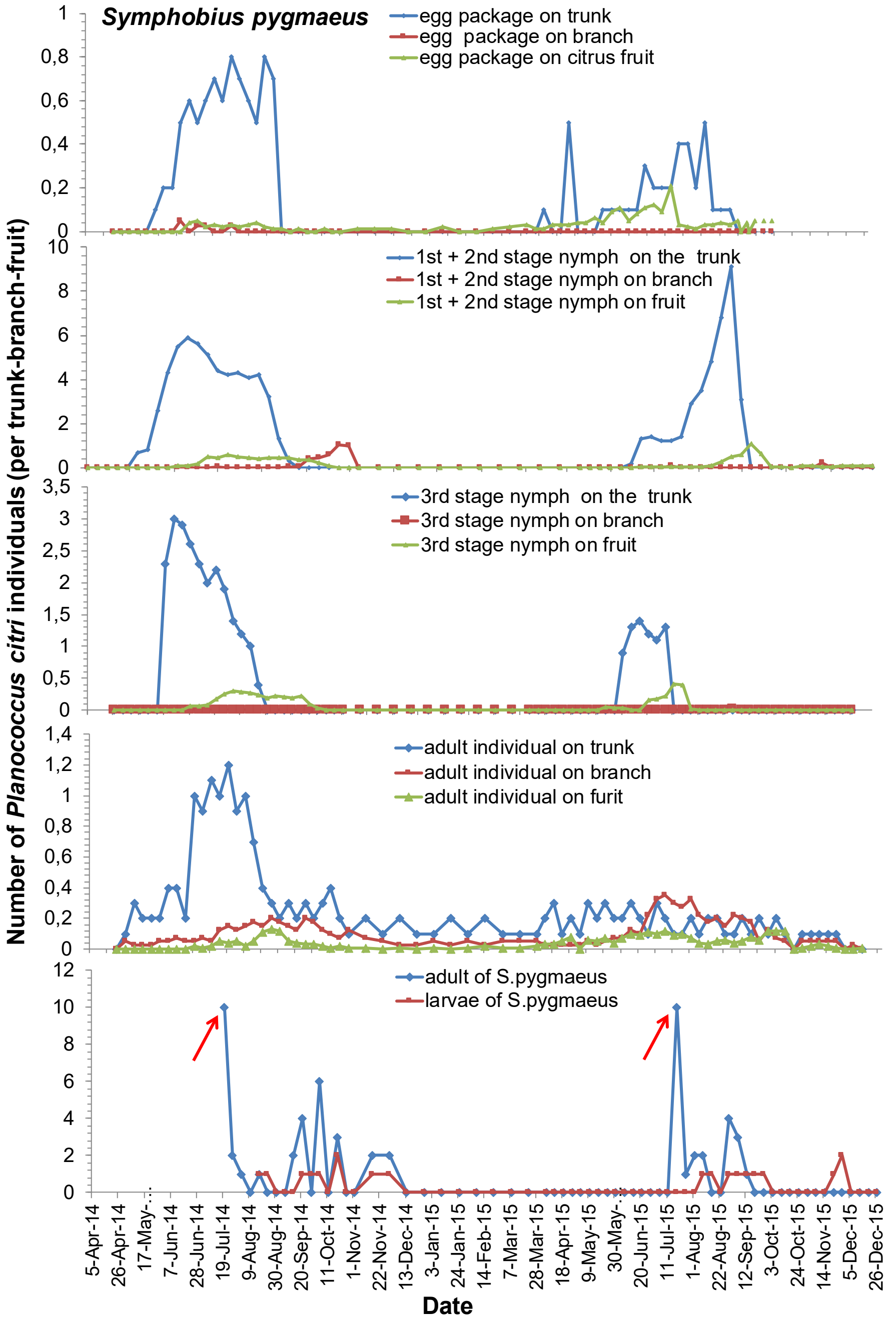

Fig. 3. Population fluctuation of *Sympherobius pygmaeus* and *Planococcus citri* in grapefruit orchard in Erzin district of Hatay province in 2014 and 2015 (arrow shows *S. pygmeous* releasing date).

between *S. pygmaues* and *S. fallax* with *P. citri* and showed the similarity between them in his study. *S. pygmaeus* has some advantages and disadvantages as a biological control. Its detection is very tricky as it is a good flyer. During the project, it was observed that it suppressed the pest successfully in another citrus orchard which is five kilometers away from project area. Commercial *Cryptolaemus montrouzieri* and parasitoid *Leptomastix dactylopii* are released to *P. citri* infected orchards by farmers every year as it cannot overwinter in our region. However, *S. pygmaeus* activity occurs in all seasons and is able to overwinter in our region. On the other hand Şimşek & Uygun (2016) determined that *S. pygmaeus* is very sensitive to insecticides. Increasing pesticide use affects it negatively and its population significantly decreases in the field condition. It proved that the predator is insufficient to control the pest at citrus orchards in regular pesticide usage process. In conclusion, it has high feeding capacity, short developmental time, high progeny, and overwintering ability in subtropical areas like Mediterranean; these features make *S. pygmaeus* an important candidate for biological solution of citrus mealybug but it should be produced in laboratory condition and should be released like other commercial natural enemies in order to obtain successful control. At that point, results of this research show that it functions as a solution in farm condition and can be used as an alternative in *P. citri* control.

Table 1. Coleoptera and Neuroptera predator species and numbers obtained by Steiner and visual control in three grapefruit orchards in Adana-Çukurova, Mersin-Alata and Hatay-Erzin from April 2014 to December 2015.

Order-Family	Scientific name	Adana	Mersin	Hatay
Coleoptera				
Coccinellidae	*Adalia bipunctata*	23	42	2
	Coccinella septempuntata	45	50	10
	Chilocorus bipustulatus	58	75	15
	Rodolia cardinalis	25	10	5
	Stethorus punctillum	21	15	5
	Stethorus gilvifron	15	12	0
	Nephus includens	10	12	0
	Scymnus spp.	36	62	15
Neuroptera				
Hemerobiidae	***Sympherobius pygmaeus***	**94**	**114**	**56**
Chrysopidae	*Chrysoperla carnea*	150	125	32
	Dichochrysa flavifrons	5	11	1
	Dichochrysa prasina	2	0	0
Coniopterygidae	*Conwentzia hageni*	32	16	0
	Semidalis aleyrodiformis	26	34	0
	Coniopteryx parthenia	15	5	0

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