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HORTICULTURAL STUDIES

RESEARCH PAPER

Banker Boxes, A Novel Release Method, Improve The Biological Control of *Planococcus citri* by *Cryptolaemus montrouzieri* and *Leptomastix dactylopii* in Pomegranate

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Abstract

A study was conducted to determine the efficacy of a predator, *Cryptolaemus montrouzieri* (Muls.) (Coleoptera: Coccinellidae) and a parasitoid, *Leptomastix dactylopii* (How.) (Hymenoptera: Encyrtidae), in biological control of citrus mealybug *Planococcus citri* Risso (Hemiptera: Pseudococcidae), a major pest in pomegranate orchards in Antalya. When the pests were observed in 2013, 10 predators and 20 parasitoids were concurrently released per tree in plots using the standard procedures. In 2014 and 2015, despite the partial control (74%) in 2013, a different method release method was used; a modified banker box. In this method, beneficial insects were established in cardboard boxes that included potato tubers infested with prey pest before release. These boxes were then placed in the orchard before pest seen. The total number of beneficials in three boxes was equal to the number of beneficials released in 2013. The banker box method gave promising results, with control of nearly 90% in both 2014 and 2015. These findings demonstrated that the banker box application increased the effectiveness of the release of beneficials and it suggested that this method should be considered for biological control of citrus mealybug in pomegranate.

1. Introduction

Pomegranate (*Punica granatum* L.) is a fruit species with a significant cultural history. Although it is mostly a tropical and subtropical fruit, it can also grow to a more limited in hot and temperate climates. Pomegranate cultivation is more than 300 kha with 3 Mt of production in the world. The top countries for the pomegranate cultivation are India, Iran, China, United States, Israel, Egypt and Spain (Melgarejo-Sánchez et al., 2015).

In pomegranate cultivation is affected by a wide range of insect pests. Citrus mealybug [*Planococcus citri* (Risso) (Hemiptera: Pseudococcidae)], Mediterranean fruit fly [*Ceratitis capitata* Wied. (Diptera: Tephritidae)], carob moth [*Ectomyelois ceratonia* (Zell.) (Lepidoptera: Pyralidae)] and honeydew moth [*Cryptoblabes gnidiella* Mill. (Lepidoptera: Pyralidae)] are important pests particularly during the ripening period. The pomegranate aphid [*Aphis punicae* Passerini (Hemiptera: Aphididae)] and ash whitefly [*Siphoninus phillyreae* (Haliday) (Hemiptera: Aleyrodidae) are important pests during the growth and flowering. Leopard moth [*Zeuzera pyrina* L. (Lepidoptera: Cossidae) also causes significant damages to pomegranate tree trunk and branches (Öztürk and Ulusoy, 2009; Öztop et al., 2010; Öztop et al., 2016; Karaca et al., 2018).

The hosts of the polyphagous citrus mealybug are mostly the citrus, but the pest harmful in other fruit species, some vegetables and ornamental plants including avocado, banana, begonia, bougainvillea, eggplant, mango, melon, mulberry, oleander, olive, peanut, pomegranate, pumpkin, vine and watermelon. Immature stages and adult females feed on fruits by sucking sap, and contribute to the development of sooty mold, severely reducing the marketable quality of the fruit. Also, their honeydew secretions support the development of other pests such as the Honeydew moth.

To control of citrus mealybug, chemical methods are generally adopted. However, chemical control of citrus mealybug is difficult because at some stages they feed between the leaves and the fruit and are inaccessible to contact insecticides. Also, resistance to some commercial insecticides has been reported (Franco et al., 2004; Franco et al., 2009; Venkatesan et al., 2016).

A potentially better option is biological control using of mass-reared and commercially available predators, *Cryptolaemus montrouzieri* (Muls.) (Coleoptera: Coccinellidae), and parasitoids, *Leptomastix dactylopii* (How.) (Hymenoptera: Encyrtidae) and *Anagyrus pseudococci*. In some cultivated plants, including cotton, citrus and guava, biological control of various mealybugs has been successfully implemented using *C. montrouzieri* (Panis and Brun, 1971; Khan et al., 2012; Kütük et al., 2014; Omkar and Kumar, 2016). The parasitoid, *L. dactylopii,* also has advantages for mealybug control including faster colonization and ability to find the host in places where the predator could not reach. The use of both beneficials in mealybug control increases the success. However, *C. montrouzieri* and *L. dactylopii* do not enter quiescence or diapause under cold temperatures (Roy and Migeon, 2010), thus they are unable to establish in the cooler temperate regions of western Europe, northern Mediterranean areas and California (except coastal areas) (Olivero et al., 2003; Hoy, 2008; Zappalà, 2010; Maes et al., 2015). Also, neither *C. montrouzieri* nor *L. dactylopii* can overwinter under climatic conditions in Turkey, so they must be released every growing season (Kütük et al., 2014).

Banker plants are hosts for the pest that can also support alternative prey or hosts for the natural enemies and are mostly used sustainable management of arthropod pests in greenhouse vegetable production (Jacobson and Croft, 1998; Schoen, 2000; Huang et al., 2011; Payton Miller and Rebek, 2018). They allow the beneficial insect to establish early, even when there is no prey or hosts in the target agroecosystem, thus frequent or high-rate of release of beneficial insects is not required (Payton Miller and Rebek, 2018).

Scientific evaluation of the use of beneficials in pomegranate plants has been quite limited. Therefore, the present study aimed to determine the potential effectiveness of *C. montrouzieri* and *L. dactylopii* in biological control of the citrus mealybug using banker boxes, a novel release method in pomegranate orchards.

2. Materials and Methods

2.1. *Cryptolaemus montrouzieri* and *Leptomastix dactylopii* rearing

Leptomastix dactylopii were mass-reared in wooden cages 55 × 35 × 45 cm (LWH) with the top covered with glass and the rear side covered by netting for ventilation. A single layer of potato tubers with sprouts infested with citrus mealybugs were placed in these cages and *L. dactylopii* adults collected from a stock culture with a vacuum pump were released into the cages. For *C. montrouzieri* mass-rearing, potato tubers infested with the citrus mealybug were likewise placed into these cages then, *C. montrouzieri* adults were released to the rear the predators. Rearing was conducted in a controlled environment room at 25 ± 1°C, 65 ± 10% RH and 16:8 h L:D photoperiod.

2.2. Experimental design

Two treatments were examined; release of both *C. montrouzieri and L. dactylopii* and a control with no beneficials released. The experiments were set-up in a randomized complete block design with four replicates plots 0.1 ha that included 66 trees. A buffer of three rows was allocated between the plots to minimize any potential dispersal of beneficials between treated and untreated plots. The 2013, 2014 and 2015 experiments were set-up in an orchard of 13-year-old *P. granatum* cv. Hicaz trees in Başköy Village in Antalya Province, Turkey. The daily temperature data was provided by the National Meteorological Service Station located approximately 12 km away from the orchard.

2.3. *Cryptolaemus montrouzieri* and *Leptomastix dactylopii* release

Adults were released directly into the orchard in 2013 following standard practice when 5% infected fruit detected. Since the proportion of infested fruits was higher than expected at the end of the 2013 growing season, a banker box release method (described below) was used in 2014 and 2015. In both methods, 10 predators and 20 parasitoids per tree were concurrently released.

The standard practice for releasing *C. montrouzieri* release involved placing 1 × 1 × 8 cm release boxes on tree trunks (1 box/tree). To release *L. dactylopii* the netting from box that contained parasitoid adults was removed and the adults were released while walking quickly around the middle are of treated plots.

For *C. montrouzieri* and *L. dactylopii* banker box release, 20 × 20 × 20 cm cardboard boxes with 10 × 10 cm windows covered with netting on four sides were used. Circular holes (1 cm) were made above each window to allow the exit of beneficials. The top of the box had a window covered transparent hard plastic sheet that permitted observation of the box contents (Figure 1). The box included two to three potato pieces (according to the size of the potato tuber) infested with mainly third instar of citrus mealybug to support *L. dactylopii* and third instar or adult females to support *C. montrouzieri*. The number of parasitoids or predators adequate for the release was collected and placed in a banker box 2 days before the planned release date. For release, boxes were hung on a branch of the tree, without direct contact with that, or any other, branch using metal wire. Adhesive gel was applied to the wire to prevent any mealybug instars spreading to the tree. Three boxes were installed in each treated plot. The release rates and dates for the three years are given in Table 1.

2.4. Sampling

Cryptolaemus montrouzieri adult population was determined at monthly intervals by a limb-tap method (Steiner, 1962). One hundred taps were made to limbs of arbitrarily selected trees. The *Cryptolaemus montrouzieri* adults collected in Steiner funnel were counted and left in the plot. *Leptomastix dactylopii* population was determined by randomly collecting five mealybug-infested fruits from each plot for examination in the laboratory. Non-target organisms were removed and the fruit placed in boxes to trap emerging adults. These boxes were checked three times per week for 3 weeks and the number of *L. dactylopii* was recorded. The proportion of mealybug-infested fruit was determined at the harvest from nine trees in the center of each plots and all fruit from these trees were assessed.

2.5. Statistical analysis

Data were processed using Jamovi version 1.6.9.0 (The Jamovi Project, 2021) for analysis and visualization. General linear models were fitted and the populations of *C. montrouzieri* and *L. dactylopii* in released plots were plotted for each of the three seasons with 95% confidence intervals, and used to compare the efficacy of different release methods. Percentage infestation data at harvest were arcsine transformed and mean separations were done on the fruit infestation rates using a paired t-test at P = 0.05. Biological efficacy was calculated using Abbott's formula (Abbott, 1925).

3. Results

During the first-year experiments conducted with the standard release method, the mean daily temperatures in June, July and August were 24.3,

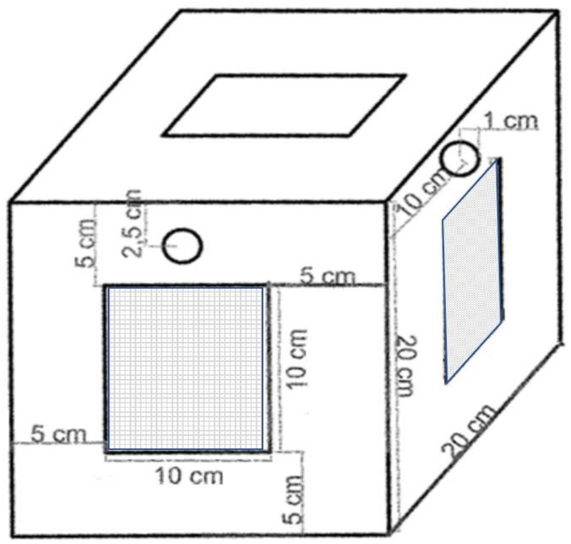

Figure 1. Banker box.

Table 1. Release rate and date of *Cryptolaemus montrouzieri* (*Cm*) and *Leptomastix dactylopii* (*Ld*) against *Planococcus citri* on pomegranate.

Year	Release method	Release rate	Release Date
2013	Standard	10 *Cm* adults and 20 *Ld* adults/tree*	9 July 2013
2014	Banker box	220 *Cm* adults/box and 440 *Ld* adults/box (3 boxes for each beneficial per treated plot)	10 June 2014
2015	Banker box	as above	18 June 2015

* The number of beneficial released per plot is the same for all years.

27.8 and 28.9ºC, respectively. Similar temperatures were occurred in the years when the banker box release method was applied. These temperatures were 24.3, 26.6 and 28.0ºC for 2014, and 22.8, 27.6 and 28.3ºC for 2015.

Although buffers were allocated between the treated and control plots, small number of predator and parasitoids were observed in the untreated plots, but the numbers can be ignored. In treated plots, the *C. montrouzieri* populations increased from the first to the last observation days in all years (Figure 2). The pattern of increase of *C. montrouzieri* was distinctly different between 2013 and the subsequent two years when the banker boxes were used. There was a clear lag in population increase in 2013; in August and September, the predator population was significantly lower than subsequent years. However, by the end of the growing season the populations in the three years were similar. The highest predator numbers were 11.3, 12.3 and 14.3 adults per sampling in October 2013, 2014 and 2015, respectively (Figure 2).

For *L. dactylopii* the response was similar by not quite as distinct, although a lag in its population development was clearly evident in the 2013 data (Figure 2). There were 2.25, 3.25, and 3.75 parasitoids per five infested fruits in August 2013, 2014 and 2015, respectively. With a similar difference in September at 3.25, 6.75 and 5.25 parasitoids, respectively. At the final assessment, the parasitoid populations were 6.75, 7.75 and 7.75 parasitoids, respectively. So, as with *C. montrouzieri*, the end of seasons populations in three years were similar.

The proportion of infested fruit were 4.1 and 15.5% in treated and control plots in 2013 (P = 0.001), indicating 73.6% control efficacy. With the banker box release in 2014 and 2015, proportion of infested fruit were 1.9 and 2.0%, respectively. Compared to 16.9 and 18.2% in control plots, giving 88.8 and 89.0% control efficacies, respectively (Table 2).

Figure 3 shows the clear benefit of banker box release in terms of high beneficial numbers and lower damage to fruit. With banker box release there was a clear decline in damaged fruit with increasing numbers (particularly in 2014), whereas this was not evident with release the standard practice (2013). Low numbers of beneficials and highly variable response is consistently with the lag evident in their population increase (Figure 3).

4. Discussion

This study aimed to determine the efficacy of the release of beneficials (10 predators and 20

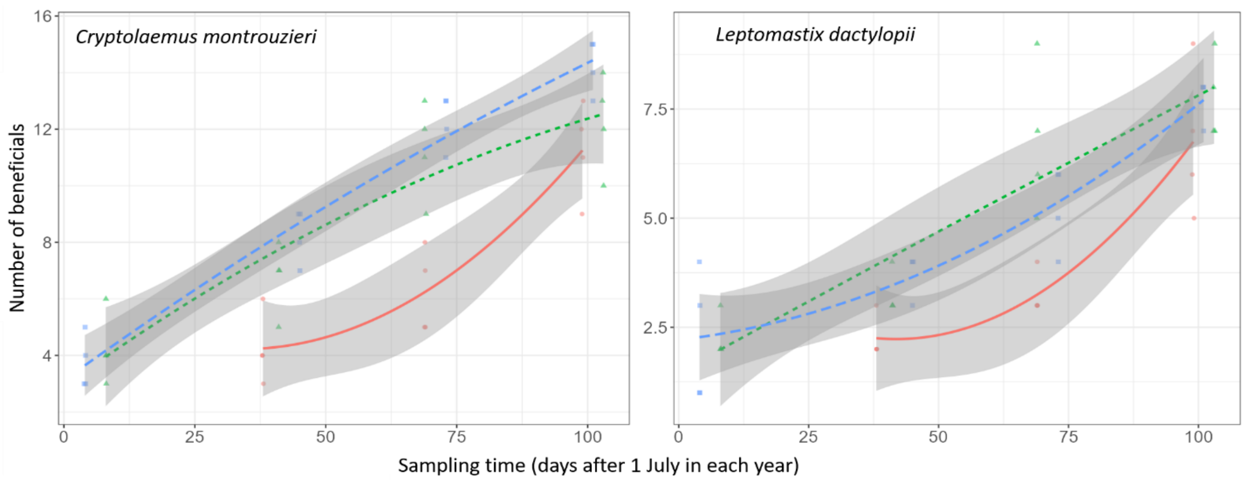

Figure 2. Numbers of *Cryptolaemus montrouzieri* and *Leptomastix dactylopii* throughout the growing season having been released by standard practice in 2013 and with banker boxes in 2014 and 2015 (Lines are quadratic general linear models fits surrounded by 95% confidence intervals).

Table 2. Mean proportion of fruit with beneficials and mealybug (%, mean ± SE) at harvest and consequent control efficacies (corrected with Abbott's formula).

Year	Fruit infestation (%) in beneficials released plots	Fruit infestation (%) in control plots	Efficacy (%)
2013	4.1 ± 0.47 a	15.5 ± 1.02 b	73.6 ± 3.14
2014	1.9 ± 0.13 a	16.9 ± 0.79 b	88.8 ± 0.37
2015	2.0 ± 0.17 a	18.2 ± 0.49 b	89.0 ± 1.16

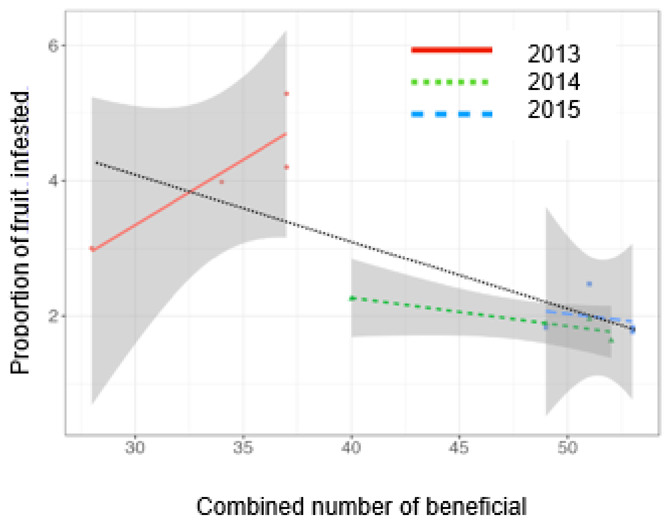

Figure 3. Proportion of fruit infested (damaged) with mealybug versus the combined number of beneficials (*Cryptolaemus montrouzieri* and *Leptomastix dactylopii*) that had been released by standard practice in 2013 and with banker boxes in 2014 and 2015 (The colored lines are simple general linear models fits surrounded by 95% confidence intervals within each year. The dotted line is the linear regression across all years emphasizing the increased number of beneficials and reduced fruit damage with banker box release in 2014 and 2015).

parasitoids) against pomegranate mealybug. While 73.6% control efficacy was obtained with release by standard practice, efficacy increased to 88.8 and 89.0% in 2014 and 2015, respectively, when banker boxes were used. Similar success has been reported using *C. montrouzieri* alone or combined with a parasitoids, such as *L. dactylopii*, in control of various mealybug species in croton (Copland et al., 1985; Afifi et al., 2010), citrus (Katsoyannos, 1996; Olivero et al., 2003; Moore and Hattingh, 2004; Rahmouni and Chermiti, 2013; Erkilic et al., 2015), grape (Mani and Thontadarya, 1988; Mani and Krishnamoorthy, 2008) and tobacco (Gautam et al., 1988). However, there appears to be no published study on the use of these beneficials in combination in pomegranate. Mani and Krishnamoorthy (2000) found that *L. dactylopii* and *Coccidoxenoides perminutus* Girault (Hymenoptera: Encyrtidae) effectively suppressed mealybug populations in August within a month of parasitoid activity in southern India. They reported high efficacy with parasitoids but this might not be the same in Antalya under its cooler conditions. In the warmer climate of their study, parasitoids are able to survive through winter and increase quickly in the spring (Krishnamoorthy, 1990).

Although somewhat promising results were obtained in in the first year of the present study using the standard release practices, 4% fruit infestation would still lead to unacceptable economic losses. Also, with this release method there is insufficient time for the populations of the beneficials to increase given the threshold number of pests were observed in July. In October, despite the increase in the beneficials inhibiting the mealybugs, the damage to the fruit already caused by the pest would lead to unacceptable quality reduction. It has been reported that *C. montrouzieri* provide efficacy from 6 weeks to 3 months (Srinivasan and Sundara Babu, 1989; Afifi et al., 2010). For citrus, the recommendation is to release *C. montrouzieri* and *L. dactylopii* when 8% tree or fruit are found to be infested in May or June in Türkiye (Erkilic et al., 2015). This means biocontrol agents do have enough time to increase in population in citrus. However, the flowering is extended in pomegranates and fruit set occurs about a month later than in citrus (Öz Atasever et al., 2011; Albrigo et al., 2019). Thus, mealybugs appear in fruits towards the end of June. This delay in fruit set means the rate of increase in the population of beneficial is insufficient. Also, in some plants such as in citrus, it is easy to detect nymphs and overwintering females on the trunks and their eggs in the cottony ovisacs. However, overwintering mealybugs in pomegranate occur mostly under the bark and difficult to detect, so it is not easy to determine as suitable release date.

To prevent mealybug damage, it is necessary to suppress the pest population in August, when the pest population is the highest and leads to sooty mold development. One strategy to solve this problem would be to use repeated releases (Erkilic et al., 2015) or higher release rates as suggested for croton (50 adult per tree) (Afifi et al., 2010). However, the additional costs involved are unlikely to be acceptable to growers. Thus, the banker box system, which is based on potato tubers infested with pests, similar to the banker plant system used for the control of pests especially in greenhouse vegetable cultivation (Osborne et al., 2005; Payton Miller and Rebek, 2018), was developed to obtain intensive beneficial population in the orchards.

Banker boxes were deployed in the second and third years of the present study achieving higher numbers of *L. dactylopii* in August and September than with release by standard practice in the first year. Similar results were also obtained for *C. montrouzieri*. Although the similar beneficial populations were seen at the end of season with both release methods, *L. dactylopii* and *C. montrouzieri* populations increased faster to a higher number with banker box release in the middle of season avoiding the lag seen with release by standard practice. The efficacy of the banker box release method was nearly 90% in 2014 and 2015.

This study has demonstrated for the first time that a banker box method would be beneficial for the suppression of the citrus mealybug populations overcoming some of the challenges of using biological control in pomegranates. Further studies are needed to optimize release rates.

5. Conclusions

The citrus mealybug is an important insect pest in pomegranate growing areas especially in Mediterranean countries. Despite using exotic natural enemies, *C. montrouzieri* and *L. dactylopii* in citrus growing, there are no studies on the efficacy of released beneficials in pomegranate. Standard release of *C. montrouzieri* and *L. dactylopii* increased control efficacy. However, based on the result of this study, the novel banker box release method is likely to increase the control efficacy of these beneficials but being able to establish their population about one month earlier than the standard approach.

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