Muştu and Tatar
Egyptian Journal of Biological Pest Control (2024) 34:52
https://doi.org/10.1186/s41938-024-00809-0

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Co-parasitization effect of *Anagyrus pseudococci* (Girault) and *Coccidoxenoides perminutus* Girault (Hymenoptera: Encyrtidae) on the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) and their intrinsic interspecific larval competition

Murat Muştu[1*] and Nuraynurgül Tatar[1]

Abstract

Background The vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) is a cosmopolitan species that causes economic damage to grapevines, especially in Mediterranean countries, South Africa, North and South America and Europe. In this study, the co-parasitization effect of *Anagyrus pseudococci* (Girault) and *Coccidoxenoides perminutus* Girault (Hymenoptera: Encyrtidae) on the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) and their intrinsic interspecific larval competition were investigated under laboratory conditions.

Results In non-choice experiments, the highest parasitism value (11.75 ± 0.87) was obtained on 2nd instar mealybug nymphs in containers with only *C. perminutus*, while the highest parasitism value (11.20 ± 0.59) in choice experiments was obtained on female mealybugs in containers with both parasitoids. It was determined that *C. perminutus* parasitized the 1st, 2nd and 3rd nymph stages, while *A. pseudococci* parasitized the 2nd, 3rd nymph and female stages of vine mealybug. These mean that mealybug populations can be controlled more effectively by parasitizing all mealybug stages in the environment when parasitoids are together. In intrinsic interspecific larval competition experiments on the mealybugs 2nd and 3rd nymph stages, where both parasitoids can parasitize, it was determined that *C. perminutus* generally won the competition, even though *A. pseudococci* had a three-day parasitism priority. The highest *A. pseudococci* emergence rate in competition trials occurred on 2nd instar mealybug nymphs when *A. pseudococci* had 72 h parasitism priority (36.09%). In addition, head capsule width and tibia lengths of *A. pseudococci* and *C. perminutus* adults obtained from intrinsic interspecific larval competition were measured, and it was determined that the head capsule width and tibia length of adult parasitoids generally increased as the host period progressed. In addition, it has been determined that there may be some differences in the measured characteristics of the adult parasitoids obtained due to intrinsic interspecific competition compared to the control.

*Correspondence:
Murat Muştu
mmustu77@hotmail.com
Full list of author information is available at the end of the article

Conclusions Using these two parasitoids together in the biological control of vine mealybugs will allow parasitizing all mealybug nymph stages and females in the environment and provide more effective pest control.

Keywords *Anagyrus pseudococci*, *Coccidoxenoides perminutus*, *Planococcus ficus*, Co-parasitization, Encapsulation, Intrinsic competition

Background

The vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) is a cosmopolitan species that causes economic damage to grapevines, especially in Mediterranean countries, South Africa, North and South America and Europe (Garcia Morales et al. 2016). Due to its biological characteristic of living in hidden places and its body surface being covered with waxy secretions, the chance of success in chemical control methods applied against *P. ficus* decreases, and this situation generally leads producers to more intensive chemical use. For this reason, alternative control methods to chemical control are emphasized in the control of vine mealybugs and biological control is one of the most important of these methods. Different natural enemies have essential potential for biological control of vine mealybug. The most well-known of these are the encyrtid parasitoids, which have been successfully used for years in some countries where the pest is known to be a problem (Cocco et al. 2021).

One of the most essential encyrtid parasitoids of vine mealybug is *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae). *A. pseudococci* is a solitary, koinobiont endoparasitoid that prefers the third instar nymphs and unmated young females of its host (Rosen and Rossler 1966). *A. pseudococci*, actively used to control vine mealybugs (Walton and Pringle 2003), is commercially available in some countries (Anonymous 2023). It is generally recognized as an effective parasitoid, although it is occasionally reported to be ineffective in controlling vine mealybugs. For example, in vineyards in the San Joaquin Valley and some coastal areas of California, USA, it has been reported that *A. pseudococci* parasitized 65% of the vine mealybug toward the end of the season (Daane et al. 2004).

Coccidoxenoides perminutus Girault (Hymenoptera: Encyrtidae), along with *A. pseudococci*, is one of the two most essential parasitoids of the vine mealybug (Walton and Pringle 2005). *C. perminutus* is a solitary, proovigenic endoparasitoid that completes its life cycle in about four weeks (Bartlett 1978). *C. perminutus* reproduces almost exclusively via thelytoky (Bartlett 1978). Males in a population are produced sporadically and at low frequency (1.2%) (Ceballo and Walter 2004). It has been used to control *P. ficus* in South Africa and California, USA (Walton and Pringle 2003). However, in previous years, *C. perminutus* was also reported to have been used against *P. citri* in biological control programs in California, Bermuda, Chile, and Italy (Bartlett 1978). It has been reported that mass releases of *C. perminutus* can be highly successful, especially when the initial population of *P. ficus* in the vineyard is low, and in general, it can be as effective as chemicals applied in vineyards in terms of suppressing the mealybug population (Walton and Pringle 2003). Furthermore, *C. perminutus* is thought to be more effective under the bark of grapevine plants as it has a smaller structure than *A. pseudococci* (Sime and Daane 2014). On the other hand, there are some opinions in the literature that *C. perminutus* may not be sufficiently successful under field conditions due to its temperature tolerance and limitations in host stage selection (Sime and Daane 2014). However, studies have reported different results on the mealybug stage preference for *C. perminutus*. It is generally stated that *C. perminutus* prefers mealybugs in the 1–3 nymphal stage, and that its main host stage is the 2nd stage nymphs (Ceballo and Walter 2004).

In some countries, such as South Africa (Walton and Pringle 2003) and the USA (California) (Gonzalez 1998), these parasitoids were released at various times against vine mealybug, and therefore, both species can be found in some vineyards. Some researchers have reported that releasing both parasitoid species against the target pest would help determine their effectiveness (Sime and Daane 2014). However, all laboratory studies on these parasitoid species and vine mealybugs have investigated the relationship between a single parasitoid and its host. Only one study in the literature investigated the host preference and biological characteristics of these two parasitoids separately at different temperatures and reported that *C. perminutus* had a higher reproductive potential on vine mealybug than *A. pseudococci* (Sime and Daane 2014).

The present study aimed to determine the co-parasitization effect of *A. pseudococci* and *C. perminutus* on *P. ficus* and the intrinsic interspecific competition between both parasitoids. Thus, it aimed to reveal the potential of using *A. pseudococci* and *C. perminutus* together to control vine mealybugs in vineyards.

Methods

Insect cultures

Planococcus ficus individuals were collected from mulberry trees in Adana/Türkiye. The mealybugs were cultured on germinated potatoes in 3 l plastic jars tightly covered by muslin (40 mesh cm^2) mesh (0.3–0.4 cm) in the laboratory climate room.

The parasitoids: *A. pseudococci* and *C. perminutus*, were collected from mulberry trees infested with *P. ficus* colonies in Adana/Türkiye, brought to the laboratory and cultured on sprouted potatoes infested with vine mealybug. Parasitoids were produced separately for both species. To rear parasitoids, potatoes infested with mealybugs in the appropriate stage (unmated young females for *A. pseudococci* and 2nd stage nymphs for *C. perminutus*) were placed in 3 l plastic jars with a ventilation hole covered with muslin (Muştu and Kılınçer 2015). All insect stock cultures were reared in climate chambers with a temperature of 25 ± 1 °C, 60 ± 10% relative humidity, and (16:8) (light/dark) lighting conditions (Muştu et al. 2022).

Co-parasitization effect of A. pseudococci and C. perminutus on various stages of P. *ficus*

The experiments were conducted with two different methods, choice and non-choice modified from (Muştu et al. 2022). In the experiments, the 1st, 2nd, and 3rd nymphal and female stages of *P. ficus* were presented to both parasitoids for parasitism at the same time as 20 individuals each, separately (non-choice) and together (a total of 80 individuals, 20 from each stage) (choice). For the experiments, mealybug individuals were reared on sprouted potatoes placed in plastic containers (10.5 × 11.5 × 9 cm) with a ventilation hole covered with muslin. To obtain mealybug individuals for the experiments, 20 potatoes, infested with mealybug eggs, were placed in individual containers. This process was repeated daily until the experiments were completed. The mealybugs that reached the appropriate stage by molting were identified; 20 individuals were left on each potato, and the excess individuals on the sprout were removed. For the choice experiments, mealybugs were reared with the same method, and other mealybug stages were transferred to the potato sprouts in a container containing 20 nymphs of the first stage. Each newly emerged female parasitoid was released 24 h before the experiment into Petri dishes (*A. pseudococci* females with three males for mating, *C. perminutus* females alone) containing 50% honey solution for feeding. At the end of 24 h, the parasitoids were transferred into the containers with mealybugs, 1 ♀ *A. pseudococci*, and 1 ♀ *C. perminutus* together. Only 1 ♀ *A. pseudococci* or 1 ♀ *C. perminutus* was released into the containers as a control. The containers were placed in a climate cabinet at the temperature of 25 ± 1 °C, 60 ± 10% relative humidity (16:8) (light/dark). After 24 h, the parasitoid females were removed from the containers and the test containers were placed in the climate cabinet under the same conditions. At least 10 days after parasitization, mummies on the potatoes were collected, placed in Eppendorf tubes, and kept under the same conditions for emergence. Non-mummified individuals were placed in 70% alcohol and dissected at the end of the experiment. Before dissection, mealybug individuals were kept in saline for 24 h and dissected under a stereo binocular microscope. At the end of the experiment, parasitism rates, developmental times, and effective encapsulation rates of *A. pseudococci* and *C. perminutus* at various stages of vine mealybug were determined. The experiments were conducted with 20 replicates.

Intrinsic interspecific competition of A. pseudococci and C. perminutus on P. *ficus*

Based on the literature (Walton and Pringle 2005) and the results of co-parasitism trials, 2nd and 3rd instar nymphs of *P. ficus*, which both parasitoids can parasitize, were used in the experiments. Petri dishes with a diameter of 5 cm were used in parasitization studies, and mealybugs parasitized by both species were placed on sprouted potatoes in plastic containers of (10.5 × 11.5 × 9 cm). Each newly emerged female parasitoid was released 24 h before the experiment into Petri dishes (*A. pseudococci* females with three males for mating, and *C. perminutus* females alone) containing 50% honey solution for feeding.

The experiments were conducted in three separate ways based on (Muştu and Kılınçer 2015). First, using the method described in the co-parasitism trials, mealybugs that reached the appropriate period were parasitized individually in Petri dishes by both *A. pseudococci* and *C. perminutus* for two hours. Parasitized mealybugs were transferred onto new sprouted potatoes, and the containers with these potatoes were placed in the climate chamber. Second, mealybugs parasitized individually by *A. pseudococci* in Petri dishes were also parasitized by *C. perminutus* after 24, 48, and 72 h. Third, the mealybugs were first presented to *C. perminutus* for parasitism, and 24, 48, or 72 h after parasitism, they were parasitized by *A. pseudococci.* In addition, the studied nymphal stages of mealybugs were parasitized only by *A. pseudococci* or *C. perminutus* using the same methods as the control group. Mealybugs parasitized by both parasitoids or parasitized by only one parasitoid were transferred onto a new sprouted potatoes, and the containers with these potatoes were placed in a climate cabinet with a temperature of 25 ± 1 °C, 60 ± 10% relative humidity (16:8) (light/dark). Each experiment was conducted in five replicates, with 20 individuals in each replicate.

At least 10 days after parasitization, the mummified individuals in the containers were collected, transferred to Eppendorf tubes, and kept for emergence. The head capsule width and tibia length of the 3rd pair of legs of parasitoid adults obtained from each experiment were measured (Muştu et al. 2022). The measurement program Leica IM50 Image software was used for the measurements. Non-mummified individuals were placed in 70% alcohol and dissected at the end of the experiment to determine effective encapsulation rates. Before dissection, mealybugs were kept in saline solution for 24 h and dissected under a stereo binocular microscope.

At the end of the experiment, the result of the intrinsic interspecific competition between *A. pseudococci* and *C. perminutus* parasitizing vine mealybug at different time intervals and the developmental time, effective encapsulation rates, and some morphometric measurements of the individuals winning the competition were determined.

Statistical analysis

Two-way ANOVA for independent samples was used to compare the means in the non-choice trials of the co-parasitization effect of *A. pseudococci* and *C. perminutus* on *P. ficus*, and differences between groups were determined according to the Tukey multiple comparison test. The experiments in choice, repeated measures two-way ANOVA was used to compare the means, and differences between groups were determined by the Tukey multiple comparison test. One-way ANOVA for independent samples was used to compare the group means in the intrinsic interspecific competition trials of *A. pseudococci* and *C. perminutus* on *P. ficus*, and differences among the groups were determined according to the Tukey multiple comparison test. Effective encapsulation rates (EE) were determined according to the following formula (Güleç et al. 2007):

$$EE = \frac{\text{Mealybugs with encapsulated eggs only}}{\text{Total parasitized mealybug number}} \times 100$$

IBM SPSS Statistics, for windows version 22.0, was used for all analyses (SPSS 2013).

Results

Co-parasitization effect of A. pseudococci and C. perminutus on various stages of P. *ficus*

The results of the co-parasitization experiments showed that there was an interaction between parasitoid species and mealybug stages in the parameters of number of mummies and developmental time (Table 1). When the results of non-choice trials were analyzed in terms of the number of mummies, it was observed that *A. pseudococci* did not develop at all in the 1st stage nymphs of *P. ficus* and very few developed in the 2nd stage nymphs (Fig. 1a). In *C. perminutus*, no mummy formation was observed in female individuals of *P. ficus*, while the number of mummies formed in the 3rd instar

Table 1 Two-way ANOVA of co-parasitization effect of *Anagyrus pseudococci* and *Coccidoxenoides perminutus* on various stages of *Planococcus ficus*

Parameter	Source	df	MS	F	*p*
Number of mummies (non-choice)	Mealybug stages	3	29.083	8.440	< 0.001
	Parasitoid species	2	713.329	206.999	< 0.001
	Interaction (Mst x Psp)	6	730.596	212.009	< 0.001
	Error	228	3.446		
Number of mummies (choice)	Mealybug stages	3	147.717	23.703	< 0.001
	Parasitoid species	2	453.162	134.166	< 0.001
	Interaction (Mst x Psp)	6	497.362	23.703	< 0.001
	Error	171 (Ps: 57)	6.232		
Developmental time	Mealybug stages	3	71.139	41.458	< 0.001
	Parasitoid species	5	561.480	327.218	< 0.001
	Interaction (Mst x Psp)	8	90.435	52.703	< 0.001
	Error	1684	1.716		
Effective encapsulation rates	Mealybug stages	3	342.586	4.055	0.008
	Parasitoid species	2	366.530	4.339	0.015
	Interaction (Mst x Psp)	2	15.065	0.178	0.837
	Error	152	84.476		

Two-way ANOVA results of number of mummies (non-choice), number of mummies (choice), developmental time, and effective encapsulation rates of *Anagyrus pseudococci* and *Coccidoxenoides perminutus* parasitizing various stages of *Planococcus ficus* (df: degrees of freedom; MS: mean squares; Mst: Mealybug stages; and Psp: Parasitoid species)

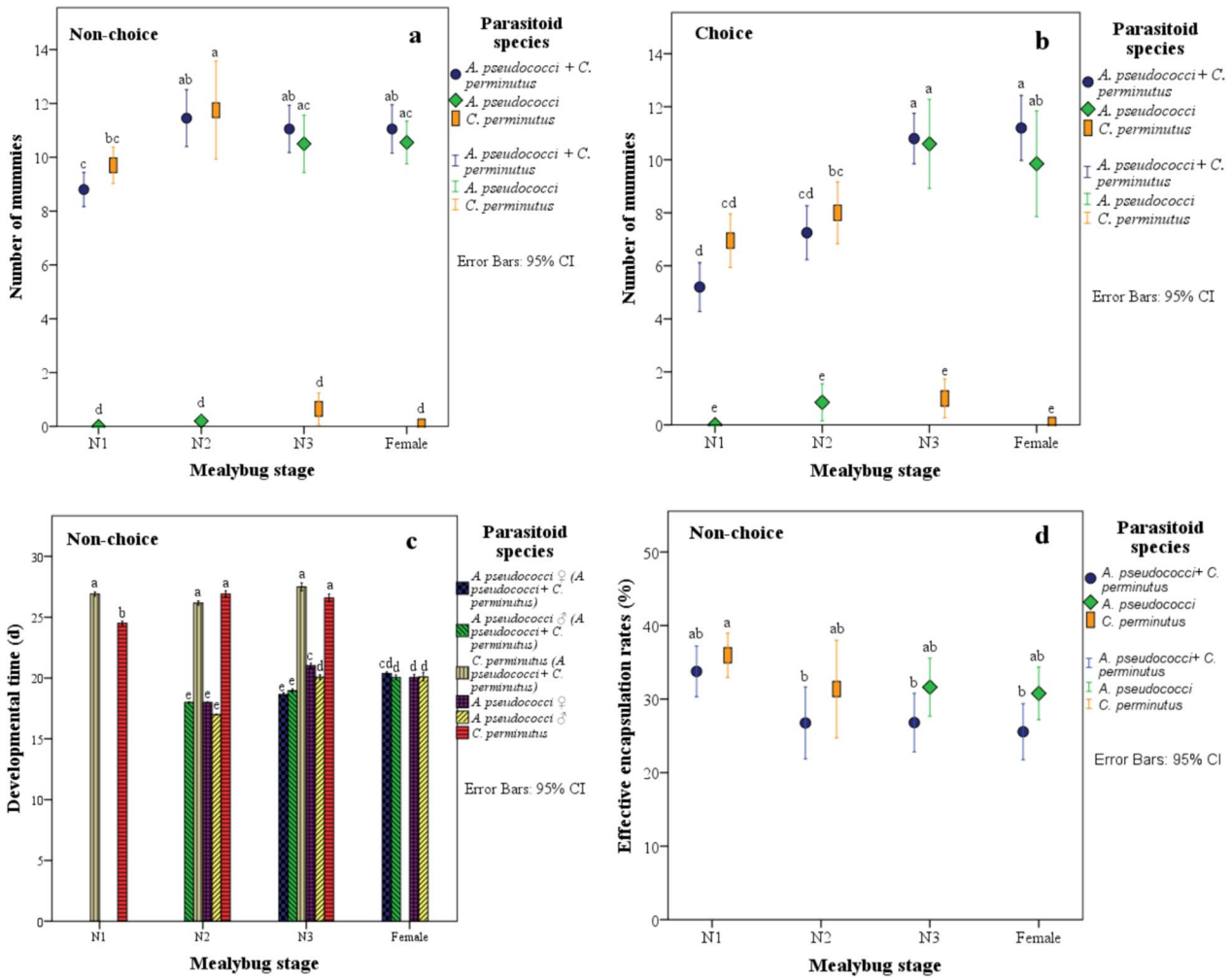

Fig. 1 Mummy numbers, developmental times, and effective encapsulation rates of *Anagyrus pseudococci* and *Coccidoxenoides perminutus* parasitizing various stages of *Planococcus ficus*. Mean ± SE mummy numbers [(**a**) (non-choice test) and (**b**) (choice test)], developmental times (**c**), and effective encapsulation rates (%) (**d**) (non-choice test) of *Anagyrus pseudococci* and *Coccidoxenoides perminutus* parasitizing various stages of *Planococcus ficus*. Different lowercase letters in the same figure are statistically different according to the Tukey test ($p \leq 0.05$)

nymphs was meager. However, when parasitoids were together, all mealybug stages were parasitized, and only the number of mummies in the 1st nymph stage was statistically lower than the number of mummies in the 2nd and 3rd nymphal and female stages (Fig. 1a).

When the number of mummies formed in the choice experiments was examined, it was determined that when *A. pseudococci* and *C. perminutus* were together, the parasitoids preferred the 3rd nymphal and female stages of the mealybug to parasitize (Fig. 1b). When parasitoids were together, the number of mummies in the 3rd nymphal and female stages of the mealybug was statistically different from the first two nymphal stages, but the number of mummies in the 2nd nymphal stage was same with the 1st nymphal stage (Fig. 1b).

When the developmental times of the parasitoids were examined, both *A. pseudococci* females and males and *C. perminutus* individuals were found to differ in their developmental time at different mealybug stages (Fig. 1c). The developmental time of *A. pseudococci* females and males increased as the mealybug stages progressed, whereas the shortest developmental time of *C. perminutus* was observed when the parasitoid developed on mealybugs in the 2nd nymph stage (Fig. 1c).

The present study showed that the interaction between various stages of mealybug and parasitoid species in terms of effective encapsulation rate was non-significant ($F_{(2,\ 152)} = 0.178$, $p = 0.837$) (Table 1). In addition, there was no difference in effective encapsulation rates whether different stages of the mealybug were parasitized

by a single parasitoid species or by both parasitoid species (Fig. 1d).

Intrinsic interspecific competition of A. pseudococci and C. perminutus on P. *ficus*

Mealybug parasitized by both parasitoids at the same time (within two hours)

Only the *C. perminutus* species emerged from the 2nd nymphal stage of *P. ficus* parasitized by *A. pseudococci* and *C. perminutus* at the same time (Table 2). Due to the values obtained, statistics could not be applied to the emergence rates of the species, but when the sexes of the emerging parasitoids were compared, it was determined that there was a difference between the emergence rates of the individuals ($F_{(5,\ 24)}=1{,}140.680$, $p<0.001$). When the developmental times of the parasitoids emerging from 2nd stage mealybugs were examined, a statistical difference was found in both species ($F_{(2,154)}=220.059$, $p<0.001$) and sex ($F_{(3,154)}=147.147$, $p<0.001$) comparisons. When the encapsulation rates of the species were compared, it was determined that the encapsulation rate in the host parasitized by both parasitoids together was higher than in the *C. perminutus* control group ($F_{(2,\ 12)}=5.209$, $p=0.024$).

There was a statistical difference between the parasitoid species ($F_{(3,\ 16)}=67.010$, $p<0.001$) and sex ratios ($F_{(5,\ 24)}=89.199$, $p<0.001$) of parasitoids emerging from mealybugs in the 3rd nymphal stage of *P. ficus*, parasitized by *C. perminutus* and *A. pseudococci* on the same day (Table 2). The developmental time of parasitoids emerging from the host was different in terms of both species ($F_{(3,\ 204)}=540.293$, $p<0.001$) and sex ($F_{(4,\ 204)}=423.138$ $p<0.001$). The encapsulation rate of the 3rd instar mealybugs parasitized by the two parasitoids on the same day was found to be the same as in the *A. pseudococci* control group but considerably lower than in the *C. perminutus* control group ($F_{(2,\ 12)}=19.345$, $p<0.001$) (Table 2).

Mealybug parasitized by C. perminutus, then multi-parasitized by A. pseudococci

It was determined that a very high rate of *C. perminutus* emerged from mealybugs parasitized by *A. pseudococci* 24, 48, and 72 h after being parasitized *C. perminutus*. The highest *A. pseudococci* emergence (10.39%) was from mealybugs in the 3rd nymph stage, which were also parasitized by *A. pseudococci* one day after being parasitized *C. perminutus* (Table 3). There was a statistical difference between the ratio of both species ($F_{(3,\ 16)}=299.725$, $p<0.001$) and sex ratio ($F_{(5,\ 24)}=214.908$, $p<0.001$) of parasitoids emerging from the 3rd nymphal stage individuals of *P. ficus* parasitized by *A. pseudococci* one day after being parasitized by *C. perminutus*. When the developmental times of the parasitoids were examined, it was found that the individuals emerging from the 3rd stage nymphs were parasitized by both parasitoids at one-day intervals were statistically different from each other and the control groups in terms of species ($F_{(3,194)}=956.287$, $p<0.001$) and sex ($F_{(5,\ 194)}=565.271$, $p<0.001$). When encapsulation rates of these mealybugs were evaluated, it was found that the encapsulation rate in the host parasitized by both parasitoids was lower than in the *C. perminutus* control group ($F_{(2,\ 12)}=10.227$, $p=0.003$).

Table 2 Parasitoid emergence rates (%), effective encapsulation rates (%), and developmental times from *Planococcus ficus* in the 2nd and 3rd nymphal stage parasitized by *Coccidoxenoides perminutus* and *Anagyrus pseudococci* at the same time (within 2 h) (mean ± standard error)

Host stage	Parasitoid species	Emergence rate of parasitoid species (%)	Duration of development (d)	Effective encapsulation (%)	Sex	Emergence rate of parasitoid sexes (%)	Duration of development (d)
2	*A. pseudococci*	0.00 ± 0.00	–	32.00 ± 6.04a	♀	0.00 ± 0.00d	–
					♂	0.00 ± 0.00d	–
	C. perminutus	100 ± 0.00	31.02 ± 0.51a*		♀	100 ± 0.00a	31.02 ± 0.51a
	A. pseudococci (control)	100 ± 0.00	17.00 ± 0.17c	28.00 ± 2.55ab	♀	17.06 ± 2.53c	18.17 ± 0.31c
					♂	82.94 ± 2.53b	16.72 ± 0.16c
	C. perminutus (control)	100 ± 0.00	28.07 ± 0.35b	15.00 ± 1.58b	♀	100 ± 0.00a	28.07 ± 0.35b
3	*A. pseudococci*	18.08 ± 6.72b*	16.71 ± 1.09d	13.00 ± 3.39b	♀	1.11 ± 1.11d	22.00 ± 0.00
					♂	16.97 ± 5.90 cd	15.83 ± 0.75d
	C. perminutus	81.92 ± 6.72a	30.30 ± 0.27a		♀	81.92 ± 6.72ab	30.30 ± 0.27a
	A. pseudococci (control)	100 ± 0.00a	19.68 ± 0.13c	11.00 ± 1.87b	♀	29.63 ± 3.48c	20.19 ± 0.23c
					♂	70.39 ± 3.48b	19.55 ± 0.16c
	C. perminutus (control)	100 ± 0.00a	27.47 ± 0.27b	35.00 ± 3.54a	♀	100 ± 0.00a	27.47 ± 0.27b

*Different lowercase letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$)

Table 3 Parasitoid emergence rates (%), effective encapsulation rates (%), and developmental times from *Planococcus ficus* in the 2nd and 3rd nymphal stage parasitized first by *Coccidoxenoides perminutus* and later by *Anagyrus pseudococci* at different time intervals (mean ± standard error)

Priority time	Host stage	Parasitoid species	Emergence rate of parasitoid species (%)	Duration of development (d)	Effective encapsulation (%)	Sex	Emergence rate of parasitoid sexes (%)	Duration of development (d)
24 ± 2 h	2	*A. pseudococci*	1.05 ± 1.05b*	12.00 ± 0.00	22.00 ± 5.39a	♀	0.00 ± 0.00d	–
						♂	1.05 ± 1.05d	12.00 ± 0.00
		C. perminutus	98.95 ± 1.05a	30.75 ± 0.21a		♀	98.95 ± 1.05a	30.75 ± 0.21a
		A. pseudococci (control)	100 ± 0.00a	17.00 ± 0.17c	28.00 ± 2.55a	♀	17.06 ± 2.53c	18.17 ± 0.31c
						♂	82.94 ± 2.53b	16.72 ± 0.16c
		C. perminutus (control)	100 ± 0.00a	28.07 ± 0.35b	15.00 ± 1.58a	♀	100 ± 0.00a	28.07 ± 0.35b
	3	*A. pseudococci*	10.39 ± 3.54c*	15.71 ± 0.61d	15.00 ± 5.70b	♀	3.28 ± 2.14d	16.50 ± 0.50de
						♂	7.11 ± 2.92d	15.40 ± 0.81e
		C. perminutus	89.61 ± 3.54b	31.97 ± 0.20a		♀	89.61 ± 3.54a	31.97 ± 0.20a
		A. pseudococci (control)	100 ± 0.00a	19.68 ± 0.13c	11.00 ± 1.87b	♀	29.63 ± 3.48c	20.19 ± 0.23c
						♂	70.39 ± 3.48b	19.55 ± 0.16 cd
		C. perminutus (control)	100 ± 0.00a	27.47 ± 0.27b	35.00 ± 3.54a	♀	100 ± 0.00a	27.47 ± 0.27b
48 ± 2 h	2	*A. pseudococci*	0.00 ± 0.00	–	17.00 ± 2.55b	♀	0.00 ± 0.00d	–
						♂	0.00 ± 0.00d	–
		C. perminutus	100 ± 0.00	32.44 ± 0.13a*		♀	100 ± 0.00a	32.44 ± 0.13a
		A. pseudococci (control)	100 ± 0.00	17.00 ± 0.17c	28.00 ± 2.55a	♀	17.06 ± 2.53c	18.17 ± 0.31c
						♂	82.94 ± 2.53b	16.72 ± 0.16c
		C. perminutus (control)	100 ± 0.00	28.07 ± 0.35b	15.00 ± 1.58b	♀	100 ± 0.00a	28.07 ± 0.35b
	3	*A. pseudococci*	0.00 ± 0.00	–	15.00 ± 3.16b	♀	0.00 ± 0.00d	–
						♂	0.00 ± 0.00d	–
		C. perminutus	100 ± 0.00	32.10 ± 0.18a*		♀	100 ± 0.00a	32.10 ± 0.18a
		A. pseudococci (control)	100 ± 0.00	19.68 ± 0.13c	11.00 ± 1.87b	♀	29.63 ± 3.48c	20.19 ± 0.23c
						♂	70.39 ± 3.48b	19.55 ± 0.16c
		C. perminutus (control)	100 ± 0.00	27.47 ± 0.27b	35.00 ± 3.54a	♀	100 ± 0.00a	27.47 ± 0.27b
72 ± 2 h	2	*A. pseudococci*	7.35 ± 2.14c*	16.33 ± 0.42c	17.00 ± 4.64ab	♀	0.00 ± 0.00d	–
						♂	7.35 ± 2.14d	16.33 ± 0.42c
		C. perminutus	92.65 ± 2.14b	30.43 ± 0.18a		♀	92.65 ± 2.14 a	30.43 ± 0.18a
		A. pseudococci (control)	100 ± 0.00a	17.00 ± 0.17c	28.00 ± 2.55a	♀	17.06 ± 2.53c	18.17 ± 0.31c
						♂	82.94 ± 2.53b	16.72 ± 0.16c
		C. perminutus (control)	100 ± 0.00a	28.07 ± 0.35b	15.00 ± 1.58b	♀	100 ± 0.00a	28.07 ± 0.35b
	3	*A. pseudococci*	5.46 ± 2.28b*	15.75 ± 0.25d	17.00 ± 4.06b	♀	0.00 ± 0.00d	–
						♂	5.46 ± 2.28d	15.75 ± 0.25d
		C. perminutus	94.54 ± 2.28a	30.24 ± 0.18a		♀	94.54 ± 2.28a	30.24 ± 0.18a
		A. pseudococci (control)	100 ± 0.00a	19.68 ± 0.13c	11.00 ± 1.87b	♀	29.63 ± 3.48c	20.19 ± 0.23c
						♂	70.39 ± 3.48b	19.55 ± 0.16c
		C. perminutus (control)	100 ± 0.00a	27.47 ± 0.27b	35.00 ± 3.54a	♀	100 ± 0.00a	27.47 ± 0.27b

* Different lowercase letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$)

Table 4 Parasitoid emergence rates (%), effective encapsulation rates (%) and developmental times from *Planococcus ficus* in the 2nd and 3rd nymphal stage parasitized first by *Anagyrus pseudococci* and later by *Coccidoxenoides perminutus* at different time intervals (mean ± standard error)

Priority time	Host stage	Parasitoid species	Emergence rate of parasitoid species (%)	Duration of development (d)	Effective encapsulation (%)	Sex	Emergence rate of parasitoid sexes (%)	Duration of development (d)
24 ± 2 h	2	*A. pseudococci*	0.00 ± 0.00	–	30.00 ± 2.24a	♀	0.00 ± 0.00d	–
						♂	0.00 ± 0.00d	–
		C. perminutus	100 ± 0.00	31.65 ± 0.20a*		♀	100 ± 0.00a	31.65 ± 0.20a
		A. pseudococci (control)	100 ± 0.00	17.00 ± 0.17c	28.00 ± 2.55a	♀	17.06 ± 2.53c	18.17 ± 0.31c
						♂	82.94 ± 2.53b	16.72 ± 0.16c
		C. perminutus (control)	100 ± 0.00	28.07 ± 0.35b	15.00 ± 1.58b	♀	100 ± 0.00a	28.07 ± 0.35b
	3	*A. pseudococci*	8.59 ± 3.32b*	13.00 ± 0.37d	11.00 ± 5.79b	♀	0.00 ± 0.00d	–
						♂	8.59 ± 3.32d	13.00 ± 0.37d
		C. perminutus	93.41 ± 1.91a	31.76 ± 0.17a		♀	93.41 ± 1.91a	31.76 ± 0.17a
		A. pseudococci (control)	100 ± 0.00a	19.68 ± 0.13c	11.00 ± 1.87b	♀	29.63 ± 3.48c	20.19 ± 0.23c
						♂	70.39 ± 3.48b	19.55 ± 0.16c
		C. perminutus (control)	100 ± 0.00a	27.47 ± 0.27b	35.00 ± 3.54a	♀	100 ± 0.00a	27.47 ± 0.27b
48 ± 2 h	2	*A. pseudococci*	0.00 ± 0.00	–	21.00 ± 1.87ab	♀	0.00 ± 0.00d	–
						♂	0.00 ± 0.00d	–
		C. perminutus	100 ± 0.00	31.37 ± 0.19a*		♀	100 ± 0.00a	31.37 ± 0.19a
		A. pseudococci (control)	100 ± 0.00	17.00 ± 0.17c	28.00 ± 2.55a	♀	17.06 ± 2.53c	18.17 ± 0.31c
						♂	82.94 ± 2.53b	16.72 ± 0.16c
		C. perminutus (control)	100 ± 0.00	28.07 ± 0.35b	15.00 ± 1.58b	♀	100 ± 0.00a	28.07 ± 0.35b
	3	*A. pseudococci*	16.28 ± 2.87c*	15.64 ± 0.16d	11.00 ± 5.10b	♀	0.00 ± 0.00d	–
						♂	0.00 ± 0.00d	–
		C. perminutus	83.72 ± 2.87b	31.97 ± 0.19a		♀	100 ± 0.00a	31.37 ± 0.19a
		A. pseudococci (control)	100 ± 0.00a	19.68 ± 0.13c	11.00 ± 1.87b	♀	17.06 ± 2.53c	18.17 ± 0.31c
						♂	82.94 ± 2.53b	16.72 ± 0.16c
		C. perminutus (control)	100 ± 0.00a	27.47 ± 0.27b	35.00 ± 3.54a	♀	100 ± 0.00a	28.07 ± 0.35b
72 ± 2 h	2	*A. pseudococci*	36.09 ± 2.25c*	14.82 ± 0.12d	22.00 ± 3.39ab	♀	3.65 ± 2.32f	16.33 ± 0.33 cd
						♂	32.44 ± 1.20d	14.63 ± 0.13d
		C. perminutus	63.91 ± 2.25b	29.30 ± 0.13a		♀	63.91 ± 2.25c	29.30 ± 0.13a
		A. pseudococci (control)	100 ± 0.00a	17.00 ± 0.17c	28.00 ± 2.55a	♀	17.06 ± 2.53e	18.17 ± 0.31c
						♂	82.94 ± 2.53b	16.72 ± 0.16c
		C. perminutus (control)	100 ± 0.00a	28.07 ± 0.35b	15.00 ± 1.58b	♀	100 ± 0.00a	28.07 ± 0.35b
	3	*A. pseudococci*	21.63 ± 2.38c*	16.94 ± 0.20d	13.00 ± 3.39b	♀	11.18 ± 4.02d	17.44 ± 0.18d
						♂	10.45 ± 1.72d	16.38 ± 0.26d
		C. perminutus	78.37 ± 2.38b	30.70 ± 0.15a		♀	78.37 ± 2.38b	30.70 ± 0.15a
		A. pseudococci (control)	100 ± 0.00a	19.68 ± 0.13c	11.00 ± 1.87b	♀	29.63 ± 3.48c	20.19 ± 0.23c
						♂	70.39 ± 3.48b	19.55 ± 0.16c
		C. perminutus (control)	100 ± 0.00a	27.47 ± 0.27b	35.00 ± 3.54a	♀	100 ± 0.00a	27.47 ± 0.27b

* Different lowercase letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$)

Mealybug parasitized by *A. pseudococci*, then multi-parasitized by *C. perminutus*

Although *A. pseudococci* had parasitism priority for 24, 48 and 72 h, it was found that *C. perminutus* adults usually emerged from the 2nd and 3rd stage mealybug nymphs parasitized by both parasitoids. The highest *A. pseudococci* emergence (36.09%) was from mealybugs in the 2nd nymph stage, which were also parasitized by *A. pseudococci* 72 h

after being parasitized *C. perminutus* (Table 4). Parasitoids emerging from *P. ficus* individuals in the 2nd nymph stage parasitized by *C. perminutus* three days after *A. pseudococci* were statistically different than the control groups in terms of species ratios ($F_{(3, 16)}=380.928$, $p<0.001$) and sex ratios ($F_{(5, 24)}=575.349$, $p<0.001$). When the developmental times of parasitoids emerged from these mealybugs were analyzed, it was determined that there was a difference between the developmental times of parasitoid species ($F_{(3, 169)}=557.740$, $p<0.001$) and the developmental times of different sex groups ($F_{(5, 167)}=341.362$, $p<0.001$) (Table 4). In addition, although there was a statistical difference between the effective encapsulation rates of the host parasitized by different parasitoid groups ($F_{(2, 12)}=6.195$, $p=0.014$), the encapsulation rate of *P. ficus* in the 2nd nymph stage parasitized by both parasitoids was not different from the encapsulation rate of the host parasitized by a single parasitoid species.

Some morphometric measurements of *A. pseudococci* and *C. perminutus* adults

When the head width and tibia lengths of parasitoids emerging from hosts parasitized only by *A. pseudococci* or only by *C. perminutus* or as a result of intraspecific competition between both parasitoid species were examined, it was observed that the head width and tibia length of parasitoids emerging from mealybugs increased as the nymphal stage of *P. ficus* progressed (Table 5). When the 2nd and 3rd nymphal stages were examined separately, there was no statistical difference between the head widths or tibia lengths of *A. pseudococci* females emerging due to competition and the control group *A. pseudococci* females emerging from the same mealybug stage. However, when the tibia length of *A. pseudococci* males was analyzed, a statistical difference was found between the tibia lengths of all *A. pseudococci* males emerging from both intrinsic interspecific competition and control groups ($F_{(3, 152)}=288.038$, $p<0.001$).

It was determined that there was no difference in both head width and tibia length measurements between the *C. perminutus* adults emerging from the intrinsic interspecific competition or control groups in the 2nd nymphal stage of the mealybugs (Table 5). In contrast, while in the 3rd nymphal stage, both head width ($F_{(3, 343)}=430.043$, $p<0.001$) and tibia length ($F_{(3, 343)}=305.190$, $p<0.001$) of the adults emerged from the control group were longer than those emerged from the intrinsic interspecific competition.

Discussion

This study showed that the biological stages of *P. ficus* preferred for parasitization by *A. pseudococci* and *C. perminutus* are significantly different. It was determined that *A. pseudococci* did not develop at all in the 1st instar nymphs of its host and developed extraordinarily little in the 2nd instar nymphs. When *A. pseudococci* parasitized 2nd instar nymphs, it was observed that male individuals usually developed from the host at this stage. However, *A. pseudococci* mostly preferred to parasitize the 3rd instar nymphs and females of *P. ficus*. Previous studies have shared similar findings with the results we obtained. Islam et al. (1997) reported that while no mummies were formed in the 1st stage nymphs of *P. citri*, parasitized with *A. pseudococci*, mummies were formed in the 2nd and 3rd stages of mealybugs within eight to eleven days. Güleç et al. (2007) found that *A. pseudococci* laid eggs mostly on *P. ficus* nymphs aged 21 days and not on nymphs aged nine days, but showed feeding behavior on them from the host.

In this study, no mummy formation was observed in female individuals of *P. ficus* offered to *C. perminutus*, while the number of mummies formed in 3rd instar nymphs was meager. On the other hand, *C. perminutus* mostly preferred 2nd instar vine mealybug nymphs, while it was found to develop very well on 1st instar nymphs. In previous studies, there are different reports on the host

Table 5 Head width and tibia length of adult parasitoids emerging from *Planococcus ficus* at various stages parasitized by *Anagyrus pseudococci* and *Coccidoxenoides perminutus* (mean ± standard error) (μm)

Mealybug stage	*A. pseudococci*				*C. perminutus*	
	♀		♂		♀	
	Head width	**Tibia**	**Head width**	**Tibia**	**Head width**	**Tibia**
N_2	363.60 ± 4.65b*	341.47 ± 5.46b	354.44 ± 1.48b	284.08 ± 1.32c	350.96 ± 0.87c	232.64 ± 1.13c
N_3	480.20 ± 10.53a	393.99 ± 7.24a	424.66 ± 1.36a	324.16 ± 1.04a	391.93 ± 1.28b	291.99 ± 1.14b
N_2 (control)	398.46 ± 2.51b	318.12 ± 1.96b	363.79 ± 3.62b	265.61 ± 1.46d	351.10 ± 0.87c	229.60 ± 0.56c
N_3 (control)	481.45 ± 3.59a	401.54 ± 3.03a	427.75 ± 1.25a	294.44 ± 1.54b	396.99 ± 1.77a	336.30 ± 6.75a

* Different lowercase letters in the same column are statistically different according to the Tukey test ($p \leq 0.05$)

stage parasitized by *C. perminutus*. Ceballo and Walter (2004) reported that *C. perminutus* can parasitize all nymphal stages of *P. citri* except the female stage, but it mainly prefers 2nd stage hosts. Most of the eggs laid on 2nd stage mealybugs (82%) reached the adult stage, while only a few of the eggs laid on 1st and 3rd stages (about 5% each) thrived. Joyce et al. (2001) found that *C. perminutus* prefers all stages of *P. ficus*, but the 2nd, 3rd and 4th stages of mealybugs are more suitable for the parasitoid than the 1st stage nymphs, and the parasitoid does not feed on the host. Sime and Daane (2014) reported that *C. perminutus* can parasitize all stages of *P. ficus* without making a clear preference among the stages. The results obtained from this study were similar to the ones obtained by Ceballo and Walter (2004). In this study, only the number of parasitoids developed from 1st instar nymphs was higher than the rate reported by Ceballo and Walter (2004). However, when both parasitoid species were released together into an environment where all stages of mealybugs were present, all mealybug stages parasitized at a high rate in the environment, unlike when only one parasitoid species was released.

In the study, when the developmental times of *A. pseudococci* and *C. perminutus* were compared, it was observed that *A. pseudococci* completed its development in a considerably shorter time than *C. perminutus*. When the developmental times of the parasitoids were considered separately, it was determined that there were differences between the developmental times of the parasitoids according to sex and host stage differences, and there were also slight differences between the developmental times of the parasitoids obtained from the containers with two parasitoids together and the individuals obtained from the control groups with a single parasitoid species. Rosen and Rössler (1966) reported that *A. pseudococci* produced a maximum of 65 offspring per female on *P. citri* (Risso) at 26 °C and completed its life in 17–18 days on average. Güleç et al. (2007) determined the average female developmental time of *A. pseudococci* in 15- and 21-day-old individuals as 17.7 ± 0.39 and 16.65 ± 0.25 days, respectively, and the average male developmental time as 16.85 ± 0.29 and 15.25 ± 0.09 days under 28 °C temperature, 60–65% RH and 16:8 (light/dark) conditions. Ceballo and Walter (2004) reported the developmental time of *C. perminutus* on different nymphal stages (N1, N2, and N3) of *P. citri* as 25.5 ± 0.65, 27.5 ± 0.87, and 28.1 ± 0.78 days, respectively, under 28 °C temperature, 75 ± 5% RH and 12:12 (light/dark) conditions. Walton and Pringle (2005) reported that the developmental time from egg to adult of *C. perminutus* on *P. ficus* at five different temperatures (18, 20, 25, 27, and 30 °C) ranged from 27.98 days to 82.29 days depending on the temperature and the value at 25 °C was 31.19 days. Kurt and Karaca (2016) reported the developmental time of *C. perminutus* on *P. citri* as 15.975 ± 0.352 days under 25 ± 1 °C temperature and 60 ± 5% RH and (16:8) light/dark conditions. Although the developmental time value reported by Kurt and Karaca (2016) is quite unusual, it is seen that the values given in the literature regarding the developmental times of both parasitoids may differ from each other, even though they were studied under similar conditions. In this study, it was observed that there could be differences of 3–5 days between the developmental periods of *C. perminutus* individuals.

It was determined that the effective encapsulation rates of *A. pseudococci* and *C. perminutus* were generally close to each other (25–35%). However, they varied according to the host stage and parasitoid species differences, but the effective encapsulation rate when the two parasitoids were statistically lower than the control groups. No studies on the encapsulation rate of *C. perminutus* were found in the literature. However, Ceballo and Walter (2004) reported that the hemolymph of individuals parasitized by *C. perminutus* contained some granular or spherical structures, a possible sign of encapsulation. Blumberg et al. (1995) reported that the effective encapsulation rate of *A. pseudococci* in *P. ficus* was 7.7%. Güleç et al. (2007) reported the effective encapsulation rates of *P. ficus* in 15 (3rd instar nymph) and 21 days old (unmated female) individuals as 24.82 and 37.50%, respectively. The values obtained from this study are similar to those reported by Güleç et al. (2007).

According to the co-parasitization trials, although the host stage preferences of *A. pseudococci* and *C. perminutus* were significantly different, intrinsic interspecific competition trials were planned to reveal the competition that could occur on the host stages that both parasitoids could parasitize, and it was determined that *C. perminutus* generally won the intrinsic interspecific competition in all combinations formed as a result of the trials. Even in the experiment where *A. pseudococci* had parasitism priority for three days, mostly *C. perminutus* emerged from the host mealybug at both the 2nd nymph (63.91 ± 2.25) and 3rd nymph (78.37 ± 2.38) stages. Although this gives a clue to what might be encountered in nature, it should be remembered that parasitoids parasitize mealybugs individually and obligatorily in intrinsic interspecific competition trials. According to the co-parasitization trials, mealybugs in the 3rd nymph stage were preferred by *C. perminutus*, and *A. pseudococci* mealybugs in the 2nd nymph stage at low rates.

Given that various stages of the host coexist in nature, two parasitoids are unlikely to parasitize the same host. There are few studies on intrinsic interspecific competition of mealybug parasitoids in the literature. Muştu and Kılınçer (2015) examined the parasitism competition

between *A. pseudococci* and *L. dactylopii* parasitizing *P. ficus* on the same and different days. They reported that 68.63% of *L. dactylopii* and 31.37% of *A. pseudococci* emerged from the host parasitized by the two parasitoid species on the same day, and the species with parasitism priority usually won the competition. Pijls et al. (1995) examined the competition between the parasitoids *A. lopezi* and *A. diversicornis* of *P. manihoti* and reported that when both parasitoids parasitized the host within two hours, 80% *A. lopezi* adults emerged from the hosts. Researchers also reported that when the host was parasitized first by *A. lopezi* and 24 h later by *A. diversicornis*, there was non-significant increase in *A. lopezi* emergence, but when the host was parasitized first by *A. diversicornis* and 24 h later by *A. lopezi*, *A. diversicornis* emergence from the host increased to 50% (Pijls et al. 1995).

Varied factors that determine the outcome of competition between parasitoids. One of them is the hatching time of parasitoid eggs. In endoparasitoids, the larva whose eggs hatch first usually wins the competition within the host (Mackauer 1990; Godfray 1994). Another is the parasitism priority of the parasitoid. In competition between parasitoids, the parasitoid that parasitizes the host first usually wins the competition (Mills 2003). However, despite the parasitism priority of *A. pseudococci* in this study, *C. perminutus* generally won the intrinsic interspecific competition. Another factor is the encapsulation rate. However, the study found no significant difference between the encapsulation rates of the two parasitoids. Super-parasitism is another factor affecting the outcome of competition, though. However, in the present study, super-parasitism was avoided to the maximum extent, and parasitoids were removed as soon as the hosts were parasitized. The study determined that the head capsule width and tibia length of *A. pseudococci* and *C. perminutus* adults generally increased as the host period progressed. The literature has little information about the morphological characters of *A. pseudococci* and *C. perminutus*. Bugila (2014) reported that the mean tibia length of female parasitoids emerging from *P. ficus* females parasitized by *Anagyrus* sp. nr. *pseudococci* was 0.58 ± 0.005. In this study, the tibia length values of *A. pseudococci* females obtained from both competition and control groups were lower than those reported by Bugila (2014). However, this difference is thought to be related to the parasitized host stage and size. Also, this study found that when both parasitoids parasitized the host, there were differences in the measured characteristics of the developing parasitoids compared to the control. According to the results obtained, there was no change in *A. pseudococci* females; there was an increase in the tibia length of *A. pseudococci* males and a decrease in the measured characters of *C. perminutus* individuals. Harvey et al. (2013) reported that in a super-parasitized or multi-parasitized hosts, adult size could sometimes decrease or increase, as can some biological characteristics of the parasitoid that wins the competition.

Conclusions

In this study, the co-parasitization effect of *A. pseudococci* and *C. perminutus*, parasitoids of *P. ficus*, one of the most important pests of vineyards, and intraspecific competition between both parasitoids were determined. As a result, it was revealed that there might be a meager amount of competition between the both parasitoid species on *P. ficus*, as the host stages preferred by the two parasitoids to parasitize differ significantly from each other. If parasitoids parasitize the same *P. ficus* individual, *C. perminutus* was usually found to win the competition, although *A. pseudococci* had a three-day parasitization priority. Using these two parasitoids together in the biological control of vine mealybugs will allow parasitizing all mealybug nymphal stages and females in the environment and provide more effective pest control. However, the combined use of these parasitoids must be tested under field conditions to make a definitive judgment.

Acknowledgements
This manuscript is a part of the master's thesis of second author. We are grateful to Prof. Dr. M. Bora KAYDAN and Prof. Dr. George JAPOSHVILI (Agricultural University of Georgia, Georgia) for identifications of insects.

Author contributions
MM designed the study, analyzed the data, and wrote the manuscript. NT conducted the laboratory experiments. All authors read and approved the final manuscript.

Funding
We acknowledge financial support by The Scientific and Technological Research Council of Turkey (TUBITAK; Project Number: 120O866).

Availability of data and materials
The data that support the findings of this study are available from the corresponding author.

Declarations

Competing interests
The authors declare that they have no competing interests.

Author details
[1] Faculty of Agriculture, Department of Plant Protection, Erciyes University, Kayseri, Türkiye.

Received: 18 March 2024 Accepted: 17 July 2024
Published online: 03 September 2024

References

Anonymous 2023. Entocare. https://entocare.nl/biological-control-mealybugs/anagyrus-pseudococci/?lang=en Accessed 12 March 2024
Bartlett BR (1978) Citrus mealybug. In: Clausen C (ed.), Introduced parasites and predators of arthropod pests and weeds: a World Review. US Department of Agriculture Handbook, pp 150–155

Blumberg D, Klein M, Mendel Z (1995) Response by encapsulation of four mealybug species (Homoptera: Pseudococcidae) to Parasitization by *Anagyrus pseudococci*. Phytoparasitica 23:157–163
Bugila AAA (2014) Host-parasitoid relationships of *Anagyrus* sp. near *pseudococci* (Girault), (Hymenoptera, Encyrtidae), as a basis to improve biological control of pest mealybugs (Hemiptera, Pseudococcidae). Dissertation, Universidade de Lisboa
Ceballo FA, Walter GH (2004) Life history parameters and biocontrol potential of the mealybug parasitoid *Coccidoxenoides peregrinus* (Timberlake) (Hymenoptera: Encyrtidae): asexuality, fecundity and ovipositional patterns. Biol Control 29:235–244
Cocco A, da Silva VCP, Benelli G, Botton M, Lucchi A, Lentini A (2021) Sustainable management of the vine mealybug in organic vineyards. J Pest Sci 94:153–185
Daane KM, Malakar-Kuenen RD, Walton VM (2004) Temperature-dependent development of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae) as a parasitoid of the vine mealybug, *Planococcus ficus* (Homoptera:Pseudococcidae). Biol Control 31:123–132
Garcia Morales M, Denno BD, Miller DR, Miller GL, Ben-Dov Y, Hardy NB (2016) ScaleNet: a literature-based model of scale insect biology and systematics. Database. https://doi.org/10.1093/database/bav118
Godfray HCJ (1994) Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton
Gonzalez D (1998) Biological control of the vine mealybug in the Coachella Valley. Calif Table Grape Comm Ann Rep 26:4
Güleç G, Kilinçer AN, Kaydan MB, Ülgentürk S (2007) Some biological interactions between the parasitoid *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) and its host *Planococcus ficus* (Signoret) (Hemiptera: Coccoidea: Pseudococcidae). J Pest Sci 80:43–49
Harvey JA, Poelman EH, Tanaka T (2013) Intrinsic inter- and intraspecific competition in parasitoid wasps. Ann Rev Entomol 58:333–351
Islam KS, Copland MJW (1997) Host preference and progeny sex ratio in a solitary koinobiont mealybug endoparasitoids, *Anagyrus pseudococci* (Girault) in response to its host stage. Biocontrol Sci Technol 7:449–456
Joyce AL, Hoddle MS, Bellows TS, Gonzalez D (2001) Oviposition behaviour of *Coecidoxenoides peregrinus*, a parasitoid of *Planococcus ficus*. Entomol Exp Appl 98:49–57
Kurt M, Karaca I (2016) Life table of *Coccidoxenoides perminutus* Girault (Hymenoptera: Encyrtidae) on *Planococcus citri* Risso (Hemiptera: Pseudococcidae). Turk J Biol Control 7(2):153–166 (**In Turkish**)
Mackauer M (1990) Host discrimination and larval competition in solitary endoparasitoids. In: Mackauer M, Ehler LE, Roland J (eds) Critical issues in biological control. Intercept, Andover, pp 41–62
Mills NJ (2003) Parasitoid interactions and biological control. Proceedings of the 1st International Symposium on the Biological Control of Arthropods (ed. R. van Driesche), Publication FHTET-03-05. USDA Forest Service, Honolulu, HI pp 108–113
Muştu M, Kılınçer N (2015) Interspecific competition between *Anagyrus pseudococci* and *Leptomastix dactylopii*, parasitoids of the vine mealybug *Planococcus ficus*. Biocontrol 60:485–493
Muştu M, Derya FN, Tarhanacı B (2022) Host stage preference and demographic parameters of *Leptomastix dactylopii* (Hymenoptera: Encyrtidae) on vine mealybug *Planococcus ficus* (Hemiptera: Pseudococcidae). Egypt J Biol Pest Control. https://doi.org/10.1186/s41938-022-00572-0
Pijls JWAM, Hofker KD, Van Staalduinen MJ, Van Alphen JJM (1995) Interspecific host discrimination and competition in *Apoanagyrus* (*Epidinocarsis*) *lopezi* and *A. (E.) diversicornis*, parasitoids of the cassava mealybug *Phenacoccus manihoti*. Ecol Entomol 20:326–332
Rosen D, Rössler Y (1966) Studies on an Israel strain of *Anagyrus pseudococci* (Girault) (Hymenoptera, Encyrtidae). I. Morphology of the adults and developmental stages. Biocontrol 11:269–277
Sime KR, Daane KM (2014) A comparison of two parasitoids (Hymenoptera: Encyrtidae) of the vine mealybug: rapid, non-discriminatory oviposition is favored when ants tend the host. Environ Entomol 43:995–1002
SPSS (2013) IBM SPSS Statistics for Windows, Version 22.0. IBM Corp, Armonk, NY
Walton VM, Pringle KL (2003) Evaluating effectiveness of mass releases of the vine mealybug (*Planococcus ficus*) parasitoid *Coccoidoxenoides peregrinus* in Western Cape Province vineyards, South Africa. In: 1st International Symposium on Biological Control of Arthropods, pp 524–532
Walton VM, Pringle KL (2005) Developmental biology of vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae), and is parasitoid *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae). Afr Entomol 13(1):143–147

Publisher's Note