Factors affecting the distribution of two mirid bugs, *Creontiades pallidus* (Rambur) and *Campylomma diversicornis* (Reuter) (Hemiptera: Miridae) and notes on the parasitoid *Leiophron decifiens* Ruthe (Hymenoptera: Braconidae)

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This study shows how altitude, levels of flowering and squaring, and plant height are associated with the distribution and infestation rate of cotton fields by two mirid bugs, *Creontiades pallidus* (Rambur) and *Campylomma diversicornis* (Reuter) (Hemiptera: Miridae) in the southeastern Anatolia region of Turkey. We also describe the relationship between these mirids and the euphorine parasitoid, *Leiophron decifiens* (Ruthe) (Hymenoptera: Braconidae). Cotton field infestation by *C. pallidus* was negatively correlated with altitude and positively correlated with density of cotton squares and flowers. In contrast, *C. diversicornis* was present in all sampled fields together with the parasitoid *L. decifiens*; there were no significant correlations between their presence and altitude. The percentage of parasitism of *C. pallidus* nymphs by *L. decifiens* was low in both cotton and alfalfa, ranging between 1.6 and 6.5%.

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1. Introduction

Cotton is attacked by several mirid species, which cause significant losses in quality and quantity of cotton at different phenological stages (seedling to boll maturity) (O'Leary 1998, Goodell & Parlier 1998). In the Middle East, *Creontiades pallidus* (Rambur) (Hemiptera: Miridae) is a well-known pest of the intermediate (early vegetative) and reproductive stages of cotton, feeding on squares and young bolls. Crop losses of up to 54% have been reported for those stages by various researchers (Stamp 1987, Nakash *et al.* 1989, Alvarado *et al.* 1998, Efil & Ilkan 2003); however, Mehdi and Mohammad (2004) reported losses as high as 82% if the pests attacked during flowering.

C. pallidus and some other *Creontiades* spp. have recently become significant pests of cotton Turkey and several other countries (Stamp 1987,

Nakash et al. 1989, Simpson et al. 2002, Efil & Ilkan 2003, Norman 2003). Possible reasons for this change may include an expanding area of cotton production (facilitated recently by irrigation) and increasing pesticide usage against primary insect pests such as tobacco thrips, Thrips tabaci Lind., (Thysanoptera: Thripidae), spiny bollworm, Earias insulana Boisduval, and cotton bollworm, Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae). These insecticide applications may result in resistance build-up in the pests against the insecticides and have negative effects on beneficial insects (predators and parasitoids) which inhabit cotton. In Australia, for example, the planting of transgenic cotton varieties containing Bacillus thuringiensis insecticidal proteins that act against Helicoverpa spp. has been accompanied by reduced insecticide usage across the industry. Because some of the Helicoverpa sprays coincidentally controlled Creontiades dilutus, the lower pesticide usage has resulted in an increase in C. dilutus populations (Simpson et al. 2002).

In contrast, *Campylomma diversicornis* (Reuter), another member of the mirid family reported in this study, is known as a natural predator of important cotton pests, feeding on eggs and newly emerged larvae of *Helicoverpa armigera*, nymphs and adults of *Tetranychus urticae* (Koch) (Acarina: Tetranychidae), and eggs of *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) (Stamp 1983, Göven & Efil 1994, Göven *et al.* 1995, Liu *et al.* 2000).

Biological control of pest mirid species by parasitoids has not been investigated in the Middle East. The known parasitoids of Creontiades pallidus nymphs belong to the tribe Euphorine, family Braconidae (Hymenoptera). The most frequently found species are in the genera Leiophron and Peristenus, which parasitize their hosts as early instar nymphs with the solitary parasitic larva usually emerging from the fifth instar nymph (Loan & Shaw 1987). Some recent efforts to explore the natural enemies of C. pallidus suggested that the predator species, Chrysoperla carnea Steph. (Neuroptera: Chrysopidae) and Nabis capsiformis Germ. (Heteroptera: Nabidae), which feed on various preimaginal stages of the pest, might be suitable for biological control (Fathipour et al. 2004). However, these nonspecific natural enemies may not result in sufficient control of *C. pallidus*, so the present study was conducted to better understand the factors affecting distribution of the parasitoid *L. decifiens* and its effects on two mirid bugs, *Creontiades pallidus* and *Campylomma diversicornis*, in cotton fields. The efficiency (% parasitism) of the parasitoid against *C. pallidus* in cotton and alfalfa fields in the southeastern Anatolia region of Turkey was also investigated.

2. Materials and methods

2.1. Survey sites and sampling

Random surveys were performed August to September in each of 2002, 2003, 2005, and 2006 in cotton fields in the southeastern Anatolia region. Total area of the six surveyed cities is $39,355 \text{ km}^2$. The outermost distance between the surveyed locations was about 150 km from north to south, 350 km from east to west. The distance between surveyed fields within the same location was changed upon presence of cotton fields and phenological similarity of the crop, ranged between 2 and 10 km. In total, 16 different locations were surveyed; at each location, 3-10 different fields with similar plant phenology were chosen for detection and counting of the pest and its parasitoids (Table 1). Fields were divided into quadrants, and twenty-five samples were taken from each quadrant with a 38 cm diameter sweep net.

The altitude and geographic coordinates of each sampled field were obtained by a standard GPS device (Garmin). In 2005 and 2006, data on plant attributes (height, number of squares and flowers) and the elevations of the sampled fields were also recorded. During these years the abundance of another possible mirid host insect for *L*. *decifiens*, *Campylomma diversicornis*, was also assessed. Collected samples were put into a plastic bag containing a paper towel to reduce humidity and taken to the laboratory in an insulated cooler. Samples were chilled for 5 minutes in a deep freezer to enable accurate counting.

On-station experiments were conducted in Akçakale and Kızıltepe to determine parasitism by *L. decifiens* on *C. pallidus* nymphs. An alfalfa

City	Location	Percentage of fields infested by <i>Creontiades pallidus</i> (number of fields surveyed)				
		2002	2003	2005	2006	
Şanlıurfa	Central			44.4 (9)	81.8 (11)	
	Harran	70 (10)	60 (10)	55.9 (9)	86.6 (15)	
	Akcakale	100 (10)	90 (10)	× /	100 (10)	
	Viransehir	60 (10)	50 (10)	42.8 (7)	70 (10)	
	Siverek		. ,	0(3)	0 (5)	
	C.pinar	100 (10)	90 (10)	100 (6)	92.3 (13)	
Adıyaman	Central	× ,	~ /	0(11)	~ /	
	Besni	0(10)		~ /		
Mardin	Kızıltepe	60 (10)	30 (10)	40 (5)	66.6 (15)	
	Nusaybin	50 (10)	60 (10)	40 (5)	62.5 (8)	
Şırnak	Cizre	60 (10)	60 (10)			
	Silopi	80 (10)	80 (10)			
Batman	Central	60 (10)	. ,			
Diyarbakır	Central	0 (10)	0(10)			
-	Çınar	0 (5)	0 (5)	0(3)	0 (5)	
	Bismil	10 (10)	0 (10)	0 (6)	0 (12)	
Total		65 (125)	52 (105)	22 (64)	66 (104)	
Mean		50. $\hat{0}\pm10.06$ 47. $\hat{2}\pm10.5$ 32. $\hat{3}\pm10.3$ 55. $\hat{9}\pm12.7$ F=0.554, df=3, 40, p=0.649				

Table 1. Summary of survey studies conducted in the southeastern Anatolia region of Turkey in 2002–2006 (F-test used for comparison of years).

field in Akçakale was sampled by sweep net and C. pallidus nymphs were collected from the net using a mouth aspirator. Since cotton plants in Kızıltepe in late August and early September were at maturity and sampling by sweep net was impractical, the sampling technique was changed to the shake-bucket method. On average, 50 plants were shaken into plastic containers covered with mesh tops $(20 \times 30 \times 50 \text{ cm})$ and taken to the laboratory. All nymphs were counted and kept individually in plastic containers $(6 \times 6 \text{ cm})$ at 28±1 °C, 65±5% R.H. and 16:8 L:D conditions. Cotton squares and small bolls were supplied as a food source to these nymphs, which were checked daily until they either became adults, or parasitoids emerged. Dead nymphs were dissected for presence of parasitoids while some live nymphs that were less mobile and therefore suspected to be parasitized, were frozen and dissected. Percent parasitism (PP) was calculated as follows:

PP = Total Parasitized Nymphs (dissected and/or emerged) x 100 / Total collected nymphs (parasitized +unparasitized).

2.2. Data analysis

Variables associated with the distributions of pests, parasitoids, plant phenology (number of flowers, plant height) and altitude of the surveyed fields were subjected to correlation analyses. Kolmogrov-Smirnov statistics were applied to the data to determine the appropriate covariance analysis. Since K-S statistics showed a normal distribution (p>0.05), relationships among the variables were determined by Pearson's correlation coefficient. Average of insect variables, plants variables and altitude of each fields were assumed as independent observations and were used in the correlation analyses to enable showing the relationships among these variables. Percentage data were converted to $\arcsin \sqrt{\%}$ to satisfy the assumption of normality before analysis. Data of percentage fields infested by C. pallidus in the years (2002, 2003, 2005 and 2006) were subjected to one-way ANOVA to compare the percentage of infested fields among the years. However incidence of Leiophron decifiens in 2005 and 2006 was compared using t-test.



Fig. 1. The relationships between *Creontiades pallidus*, *Leiophron decifiens* and *Campylomma diversicornis* in 2005 and 2006.



Fig. 2. The relationships between *Creontiades pallidus* and altitude, plant height, and number of flowers+squares in 2005 and 2006.



Fig. 3. The relationships between *Leiophron decifiens* and altitude, plant height, and number of flowers+squares in 2005 and 2006.



Fig. 4. The relationships between *Campylomma diversicornis* and altitude, plant height and number of flowers+squares in 2005 and 2006.



Fig. 5. The relationships between altitude, plant height, and number of flowers+squares in 2005 and 2006.

3. Results

3.1. Infestation ratio and distribution of *Creontiades pallidus* **and presence of** *Leiophron decifiens*

There was no significant relationship between the abundance of C. pallidus and its parasitoid L. decifiens in 2005 (Fig. 1a), however a positive significant relationship was recorded in 2006 (Fig. 1d). L. decifiens was significantly correlated with C. diversicornis numbers in both years (Figs 1b and 1e). The presence of C. pallidus was also positively correlated with the number of flowers and squares per plant as well as plant height in 2005 and 2006 (Figs 2a-f). Correlation coefficients revealed larger populations of C. pallidus at lower elevations (Fig. 2a). In contrast to C. pallidus, distribution of the parasitoid L. decifiens was not correlated with the altitude of the locations in 2005 or 2006, but its levels were positively correlated with plant phenology (Figs 3af). C. diversicornis was present in all cotton fields regardless of altitude (Figs 4a and 4d), and was positively correlated with the number of flowers+squares per plant in 2005 and 2006, respectively (Figs 4b and 4e).

As expected, there were significant relationships between plant attributes (plant height, flower and square) and altitude: plants at higher elevations were shorter with fewer flowers and squares than plants at lower elevations (Figs 5a– f).

Infestation rates for *C. pallidus* in the surveyed cotton fields of southeastern Anatolia were 50.0%, 47.2%, 32.3%, and 55.9% in 2002, 2003, 2005, and 2006, respectively (Table 1). The percentage of fields infested by *C. pallidus* and the incidence of *L. decifiens* in 2005 and 2006 were not significantly different (Table 2).

3.2. Parasitism by *Leiophron decifiens* on *Creontiades pallidus*

In late September 2005, a total of 220 *C. pallidus* nymphs were collected from cotton plants and 163 from alfalfa in on-station experiments conducted in Akçakale and Kızıltepe. Of these, only 2% to 5% were parasitized by *L. decifiens*. In 2006, 718 and 1270 *C. pallidus* nymphs were collected from cotton and alfalfa fields, respectively, for which the parasitism rate ranged from 3.1% to 4.9% on cotton and 3.1% to 6.5% on alfalfa.

City	Location	Incidence of C. diversicornis		Incidence L. decifi	e of ens
		2005	2006	2005	2006
Şanlıurfa	Central	100	100	22.2	54.5
,	Harran	100	100	88.8	93.3
	Akcakale		100	100	
	Viranşehir	100	100	57.1	70
	Siverek	100	100	33.3	80
	C.pinar	100	100	100	92.3
Adıyaman	Central	100		63.6	
-	Besni				
Mardin	Kızıltepe	100	100	80	86.6
	Nusaybin	100	100	0	37.5
Şırnak	Cizre				
	Silopi				
Batman	Central				
Diyarbakır	Central				
2	Çınar	100	100	33.3	80
	Bismil	100	100	83.3	100
Total		64 (64)	104 (104)	39 (64)	85 (104)
Mean		100 ± 0.0	100±0.0	56.1±10.3	79.4±6.4
				<i>t</i> =-1.823, df=18, <i>p</i> =0.085	

Table 2. Incidence of *Campylomma diversicornis* and *Leiophron decifiens* in the southeastern Anatolia region of Turkey in 2005 and 2006 (t-test used for comparison of the incidence of *Leiophron decifiens* between 2005 and 2006).

4. Discussion

Our surveys conducted in southeastern Anatolia revealed that about 46% of cotton fields were infested with *C. pallidus*, and this pest was more abundant at lower elevations. This altitudinal difference may be due to climatic differences (e.g., warmer temperatures during the growing season or winter) or the presence of competitors. *C. pallidus* is an important pest of cotton in Syria and Israel (Stamp 1987, Nakash *et al.* 1989); indeed, in our surveys, the locations with the highest infestations were those close to the Syrian border, where the climate is warm and dry.

The percentage of fields infested by *C. pallidus* has not differed between the years of the present study conducted; however, differences in numbers were seen between locations in the region. High populations of the pest occurred early in the development of the cotton plants, when *C. pallidus* mainly attacked squares, flowers, and in some cases small bolls. This feeding pattern could indicate that the pest has a potential to cause severe damage with economic crop losses in cotton (Stamp 1987, Nakash *et al.* 1989, Efil 2004). These results were confirmed by our findings of significant positive correlation coefficients between the number of squares and flowers and *C. pallidus*.

Our results indicate that the distribution of *L. decifiens* is not closely related to the distribution of *C. pallidus* in the surveyed locations. In contrast, we observed a significant positive relationship between the distributions of *L. decifiens* and *Campylomma diversicornis*, suggesting that this parasitoid may be better adapted to *C. diversicornis*. In addition, the relationship between plant attributes (levels of squares and flowering) and *L. decifiens* may well indicate that phenology has an important role in attracting the parasitoid, since the parasitoid may benefit from cotton pollen or nectars from squares and flowers.

In our research station's cotton and alfalfa fields, the percentage of parasitism by *L. decifiens* was low; thus, the parasitoid appears to have little influence on suppression of *C. pallidus*. A possib-

le explanation for the low parasitism rate of C. pallidus by L. decifiens could be that certain mirid populations differ in their ability to encapsulate parasitoid eggs, resulting in different susceptibilities to euphorine parasitism (Debolt 1989). Native parasitoids that have evolved with their mirid host appear to have developed search preferences for particular host species. However, they can also develop new host-parasitoid associations such as C. pallidus and L. decifiens. Similarly, Snodgrass and Fayad (1991) reported that euphorine parasitoids had little impact on reducing populations of tarnished plant bugs in Mississippi cotton. A strong host preference was demonstrated for Leiophron uniformis, which showed higher parasitism levels on Lygus hesperus in comparison to Lygus lineolaris, regardless of the habitat (Snodgrass & Fayad 1991). The higher association between C. diversicornis and L. decifiens may be explained by a longer co-evolution of these species. Campylomma diversicornis has been known to be an important natural enemy of sucking pests and lepidopteran pests of cotton in the region (Karaat et al. 1986, Göven & Efil 1994, Göven et al. 1995). And while entomological studies have been conducted in the region since 1922, C. pallidus was only recorded in the region as a cotton pest for the first time in 1995 (Uygun et al. 1995). Efil and Ilkan (2003) observed the first large population outbreak of C. pallidus; however, high damage levels on cotton were first observed in 1999.

Recently, during an exploration for exotic parasitoids of Lygus spp. in Argentina and Paraguay, Leiophron argentinensis Shaw n. sp. was collected to be reared in the laboratory. In the hope that L. argentinensis could parasitize Lygus hesperus and Lygus lineolaris, it was mass reared on these pests in the southern U.S. (Williams et al. 2003). The natural parasitism rate of the parasitoid in Argentina ranged between 0 to 37% and averaged 7%. Though relatively low percentage parasitism levels were recorded, L. decifiens might be a choice for biological control of Lygus species in North America. Adding different parasitoids may improve biological programs against pests. Our recent efforts on the ecology of L. decifiens and its parasitism efficiency and its effects on the predator, Campylomma, are adding to our knowledge of this subject.

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