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Parasitoid encapsulation in mealybugs (Hemiptera: Pseudococcidae) as affected by the host-parasitoid association and superparasitism

Abstract - The encapsulation rates recorded for four species of mealybugs, when attacked by some of their principal and accidental encyrtid parasitoids, were studied under controlled conditions in no-choice assays. The encapsulation intensity was measured as effective encapsulation (EE) a value representing the percentage of parasitized mealybugs that encapsulated all parasitoid eggs and therefore prevented parasitoid development; and as aggregate encapsulation (AE) a value representing the percentage of eggs encapsulated. None or low rates (up to 12%) of EE by *Planococcus citri* (Risso), *P. ficus* (Signoret), and *Pseudococcus longispinus* (Tagioni-Tozzetti) were recorded when parasitization was conducted by principal parasitoids. Parasitization of *P. citri*, *P. longispinus* and *Pseudococcus viburni* (Signoret) by accidental parasitoids was characterized by high rates of AE and EE (86-100% for both values), suggesting that these mealybug species are unsuitable for the development of these parasitoids. The effects of cross parasitization of allopatric and sympatric ecotypes on the encapsulation values were investigated with *P. citri* and *A. pseudococci*, from Israel, Portugal and Sicily. EE was affected by the ecotype of both the host and the parasitoid. Thus, the lowest (17%) and the highest (70%) EE were recorded when Portuguese and Sicilian of *P. citri*, respectively, were attacked by the Israeli *A. pseudococci*. Increasing egg load (superparasitism) was evident for several of the tested parasitoids when they attacked unsuitable hosts. When *P. citri* was attacked by *A. pseudococci*, EE values for superparasitized hosts were much lower than for solitary parasitized hosts. It is suggested that this oviposition strategy aims at reducing values of EE, thereby increasing offspring survival.

Key words: Mealybugs, Pseudococcidae, parasitoids, encapsulation, superparasitism.

INTRODUCTION

Encapsulation is a common defense mechanism exerted by a host insect, usually in response to invasion by parasitoids or other foreign organisms. Encapsulation is directed against internal parasitoids, and is common among arthropods and related invertebrates. In the process of encapsulation, the host hemocytes adhere to the surface of the invading object, forming a multicellular capsule-like envelope around it. The encapsulation frequently leads to the death of the parasitoid (e.g. Salt, 1970; Strand & Pech, 1995). Encapsulation may adversely affect biological control by reducing parasitoid efficacy, and thus hinders the establishment of introduced parasitoids and increasing the frequency of pest outbreaks. High levels of encapsulation may cause difficulties in mass production programs of parasitoids (Brewer, 1971; Blumberg, 1977).

The incidence of encapsulation reflects the degree of host - parasitoid adaptability, and it is believed that absence or low rate of encapsulation is the result of co-evolution of the host and its parasitoid (see Bartlett & Ball, 1966; Bouletreau, 1986; Messenger & van den Bosch, 1971). Both host and parasitoid properties affect the frequency of encapsulation of parasitoids by scale insects (Coccoidea). Host related factors include age, physiological condition, temperature and host plant. Main parasitoid related factors are the type of host-parasitoid association and the degree of superparasitism (Blumberg, 1997). Aspects of parasitoid encapsulation in mealybugs (Pseudococcidae) has been studied by Bess (1939); Nenon *et al.*, (1988); Giordanengo & Nenon, (1990); Blumberg *et al.*, (1995); and Van Driesche *et al.*, (1986).

Most encyrtids that attack mealybugs are solitary parasitoids (Noyes & Hayat, 1994) and although only a single individual can develop in a single host, egg superparasitism is quite common. In superparasitized hosts, some of the eggs may become encapsulated while one or more eggs may escape encapsulation (Nenon *et al.*, 1988; Giordanengo & Nenon, 1990).

When *Planococcus citri* (Risso) was parasitized by *Anagyrus pseudococci* (Girault), many parasitized mealybugs that contained encapsulated eggs could be detected either alone or together with live immature stage of *A. pseudococci* (Blumberg & Mendel, unpublished). Even with the high rates of encapsulation observed in Israel in insectary cultures and in the orchard, *A. pseudococci* constitutes a major mortality factor of *P. citri* in Israeli orchards and is the most common natural enemy of the mealybug (Mendel *et al.* 1999). The high levels of encapsulation raised the assumption that *A. pseudococci* may not be a principal parasitoid of *P. citri* (Blumberg *et al.* 1995).

The objectives of the present study were:

(i) To compare the effect of the host-parasitoid association on encapsulation in several mealybug species with emphasis on *P. citri*, by examining differences in encapsulation values of what is believed to be principal and accidental parasitoids.

(ii) To examine whether the association of *A. pseudococci* with *P. citri*, involving different ecotypes of the host and the parasitoid, is likely to display distinct different encapsulation levels.

(iii) To evaluate the effect of superparasitism on encapsulation incidence, suggesting that oviposition strategy of superparasitism is aimed at increasing egg survival under high encapsulation pressure, and to ascertain the frequent occurrence of *A. pseudococci* in *P. citri* population in Israel.

MATERIALS AND METHODS

The studied insects. Four mealybug species and six encyrtid parasitoids were examined. Information on their presumed area of origin and the source of the studied populations are shown in Table 1. *A. pseudococci* and *P. citri* were examined, using different conspecific ecotypes from Israel, Portugal and Sicily.

Table 1 - Mealybug and parasitoid species tested and their origin.

Studied species	**Presumed area of origin**	**Source of the studied populations**
Mealybug		
Planococcus citri (Risso)	Tropics	Lab rearing (USA), citrus orchards in Israel, Portugal and Sicily
Planococcus ficus (Signoret)	Mediterranean	Vineyards in Israel
Pseudococcus longispinus (Targioni-Tozzetti)	Australia	Lab rearing (USA)
Pseudococcus viburni (Signoret)	West Palearctic	Lab rearing (USA)
Parasitoid		
Anagyrus pseudococci (Girault)	Mediterranean	Citrus orchards in Israel, Portugal and Sicily
Anagyrus fusciventris (Girault)	Australia	Lab rearing (USA)
Anagyrus diversicornis Mercet	?	Citrus orchards in Israel
Anagyrus sawadai Ishii	East Palearctic	Citrus orchards in Japan
Leptomastix dactylopii Howard	Tropics	Lab rearing (USA)
Leptomastix epona (Walker)	West Palearctic	Lab rearing (USA)

Experimental procedures. A total of 21 host-parasitoid combinations were studied, all as no-choice tests. Each combination was examined in 6-14 replicates. A replicate consisted of 10 mated female parasitoids, 2 – 4 days after emergence and 70-80 mealybugs at the stage of late second instar larvae or young adult females. All tested mealybug species were reared on potato sprouts. The mealybugs were exposed for parasitization for 48 h at 26°C and photoperiod of 14:10 L:D. They were kept for an additional 6-8 days at the same temperature. The mealybug were transferred to a clearing solution for 24 h, and mounted for examination.

Encapsulation parameters. Two parameters were used for measuring encapsulation values: aggregate encapsulation (=AE) and effective encapsulation (=EE) (Blumberg, 1991). AE describes the percentage of encapsulated eggs observed in the parasitized mealybugs that had been examined; EE describes the percentage of the parasitized mealybugs that encapsulated all parasitoid eggs and therefore escaped parasitism, in spite of successful oviposition by the parasitoid. Data on encapsulation and

Table 2 - The studied host-parasitoid combinations divided into four groups according to the levels of encapsulation and superparasitism.

Encapsulation group	Group characterization			Parasitism characterization
	Superparasitism	AE	EE	
I	Low	Low	Low	High physiological adaptability.
II	Low	High	High	Lack or low physiological adaptability.
III	High	High	High	Poor or low physiological adaptability with unsuccessful attempt to reduce EE by increasing egg load.
IV	High	High	Low	Intermediate physiological adaptability and successful reduction of EE by increasing egg load.

Table 3 - Number of host-parasitoid combinations as related to encapsulation level, parasitoid type and origin of host and parasitoid.

Origin	Level of effective encapsulation (EE)			
	Low		High	
	Principal parasitoid	Accidental parasitoid	Principal parasitoid	Accidental parasitoid
Sympatric	4	0	0	0
Allopatric	0	1	0	8

superparasitism regarding several host-parasitoid combinations, were obtained from a recent study by Blumberg & Van Driesche (2001).

RESULTS

Encapsulation by principal vs accidental parasitoids

Encapsulation by Planococcus *spp. Planococcus citri* (the Israeli ecotype) was exposed to two of its presumed principal parasitoids, *Leptomastix dactylopii* Howard and *A. pseudococci* (the Israeli ecotype), and to four accidental parasitoids, *L.* epona (Walker), *A. sawadai* Ishii, *A. diversicornis* Mercet and *A. fusciventris* (Girault). The results (Fig. 1) show that among the principal parasitoids, AE values were nil and approximately 70% for *L. dactylopii* and *A. pseudococci*, respectively; for EE of *A.*

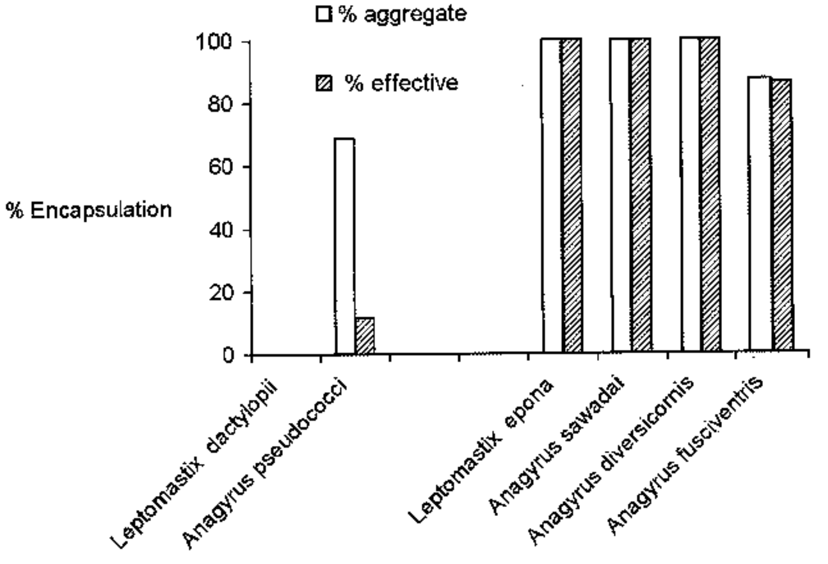

Fig. 1 - Comparison of encapsulation by *P. citri* of eggs of principal - and accidental parasitoids.

pseudococci the value was approximately 12%. The values of both AE and EE of the accidental parasitoids were high, reaching 100% in three of the host-parasitoid combinations studied. AE and EE values of *Planococcus ficus* (Signoret) were 57% and 11%, respectively, when exposed to the Israeli ecotype of *A. pseudococci* (Fig. 2).

The exposure of *A. pseudococci* of Israel, Portugal and Sicily to three respective allopatric ecotypes of *P. citri*, resulted in high values of AE ranging between 58% to 88% (Fig. 3). However, marked differences in the values of EE were registered between mealybug-parasitoid combinations; the lowest being 17% for *A. pseudococci* of Israel when attacking *P. citri* of Portugal, while the highest (70%) EE values were recorded when *A. pseudococci* of Israel attacked *P. citri* of Sicily.

Encapsulation by Pseudococcus *spp.* Encapsulation values recorded in *Pseudococcus longispinus* (Tagioni-Tozzetti) and *Pseudococcus viburni* (Signoret) when attacked by their principal parasitoids were AE= 5.7% and 42.0%, and EE= 4.0% and 33.4%, for *Anagyrus fusciventris* (Girault) and *Leptomastix epona* (Walker), respectively. When *P. longispinus* was attacked by *L. dactylopii* (an accidental parasitoid) and *L. eopna* (believed to be an accidental parasitoid) the encapsulation values were AE = 76.0% and 13.2% and EE= 68.4% and 11.2%, for the former and the latter, respectively. When *P. viburni* was attacked by *L. dactylopii* and by *A. fusciventris* (both accidental parasitoids) the encapsulation values were AE= 100% and 78.2%, and EE= 100% and 69.6%, for the former and the latter parasitoid, respectively.

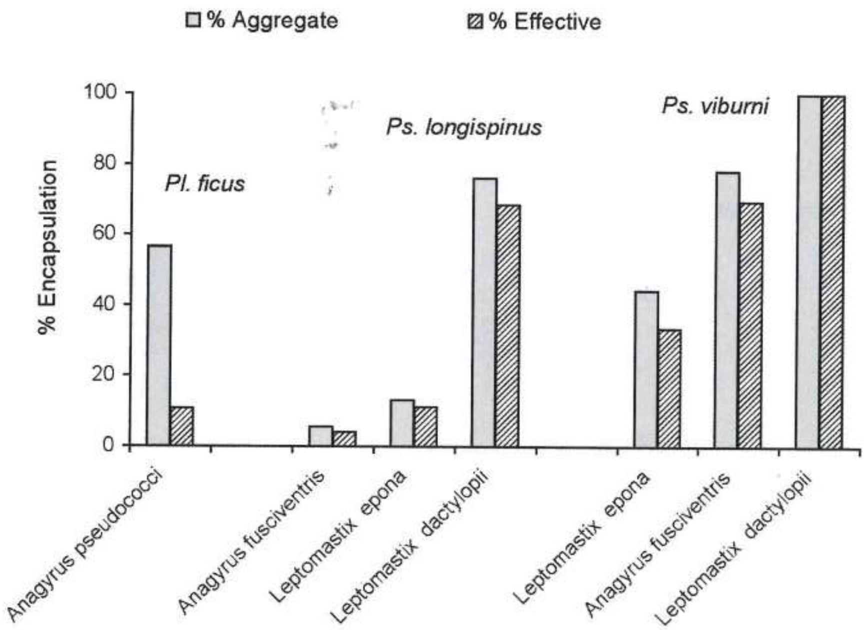

Fig. 2 - Comparison of encapsulation of principal and accidental parasitoids by three mealybug species.

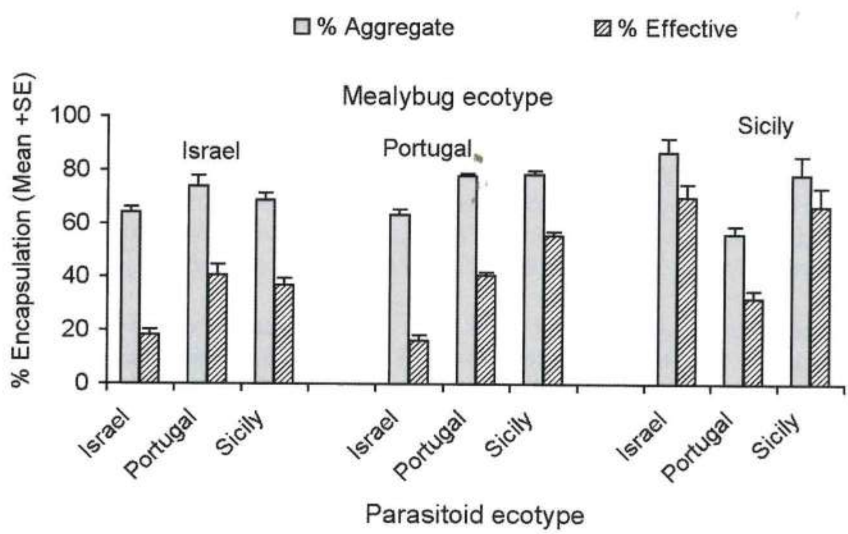

Fig. 3 - Comparison of the level of encapsulation of *Anagyrus pseudococci* eggs of Israel, Portugal and Sicily by the local ecotype of *Planococcus citri* occurring in each of the above countries.

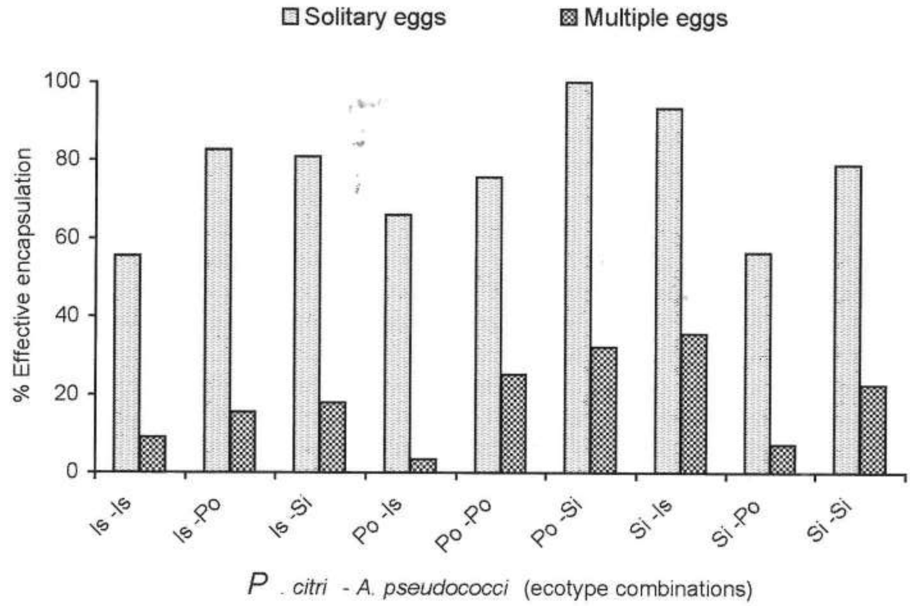

Fig. 4 - Frequency of effective encapsulation by *P. citri* parasitized with solitary and multiple eggs of *A. pseudococci.*

Effect of Superparasitism

Calculation of the encapsulation values, according to egg load, solitary *vs* superparasitized mealybugs, showed (Fig. 4) that among the nine combinations of the different ecotypes of *P. citri* and *A. pseudococci* studied, the mean EE was 76.6% for mealybugs containing solitary eggs and 18.8% for mealybugs containing multiple eggs, respectively. Similar results were obtained for the *P. ficus - A. pseudococci* combination, viz., 46% and 2.5% for solitariness and superparasitism, respectively. EE values by both tested *Pseudococcus* spp. did not show markedly difference between solitary *vs* superparasitized hosts. EE values recorded for *P. viburni* were: *L. epona* 29% and 36%, *A. fusciventris* 61% and 52%, and *L. dactylopii* 100%, for solitariness and superparasitism, respectively. EE values recorded for *P. longispinus* were: *L. epona* 13% and 0%, *A. fusciventris* 4% and 0%, and for *L. dactylopii* 62% and 61%, for solitariness and superparasitism, respectively.

DISCUSSION

The present findings reveal considerable differences in the encapsulation of the tested mealybugs between principal and accidental parasitoids. Both AE and EE values were high in combinations that included accidental parasitoids, and low in combinations of mealybug species and their principal parasitoids. The fact that none of the eggs of *L. dactylopii* was encapsulated by *P. citri,* both species probably originating in the tropics (see de Jong & van Alphen, 1989 and Bartlett, 1978), further support the idea that *L. dactylopii* is indeed most suitable for developing on *P. citri*. On the other hand the high values of AE recorded when *P. citri* was attacked by *A. pseudococci* may account to a relatively short period of association between this mealybug and *A. pseudococci.* Although the parasitoid is a Mediterranean species, AE value of *A. pseudococci* when attacked *P. ficus,* (another common Mediterranean species) was almost identical to those recorded in *P. citri*, while none of the eggs of *L. dactylopii* were encapsulated by *P. ficus* (Blumberg & Mendel, unpublished). However, in spite of the high values of AE of *A. pseudococci,* the low values of EE by both *Planococcus* spp. are the outcome of high proportion of superparasitized hosts (see below). The frequent occurrence of *A. pseudococci* in both *Planococcus* spp. populations in the field is probably due to the low EE values of this parasitoid. Our results clearly show that *P. citri* is unsuitable as host for the four accidental parasitoid species tested, *L. epona*, *A. sawadai*, *A. diversicornis* and *A. fusciventris*. This is in contrast to Noyes & Hayat (1994), who reported that specimens of *A. fusciventris* originating from California, have also been reared on *P. citri*. The encapsulation picture of the studied *Pseudococcus* spp. is less clear, although the pattern of high level of both AE and EE values of accidental as compared with principal parasitoid remains the same. However, the encapsulation values recorded for *L. epona* - probably an accidental parasitoid of *P. longispinus* were markedly lower than those recorded for *P. viburni,* although *E. epona* is thought to be the latter's principal parasitoid. These unexpected low values suggest that the determination of the origin of *E. epona* is mistaken.

Our data indicate that encapsulation rates can be markedly affected by the ecotype of both the host and the parasitoid. This was revealed by examining the encapsulation values of three allopatric ecotypes of *A. pseudococci,* by three respective allopatric ecotypes of *P. citri*. The different EE values displayed by some of the tested combinations are probably the result of a different environmental background as regards of the host and its associated parasitoid. Naturally enough, since we have tested only a single parasitoid and host population of each area so far, the findings should be treated as such. These results make some significant practical findings. The highest adaptability of host and parasitoid as regard encapsulation, exists in the combinations of the Israeli ecotype of *A. pseudococci* with mealybugs from both Israel and Portugal. On the other hand, the interaction of the Portuguese and the Israeli ecotypes of *A. pseudococci* with mealybugs from Sicily, resulted in a very high level of EE. The choosing of these parasitoid ecotypes for the control of that particular *P. citri* population may have ended with insignificant mortality in the mealybug populations or could even undermined the establishment of the parasitoid.

Concerning the effect of superparasitism, Blumberg & Luck (1990) demonstrated that multiple eggs of the encyrtid parasitoid, *Comperiella bifasciata* (Howard) were less likely to be encapsulated than solitary eggs in a California strain of the California red scale, *Aonidiella aurantii* (Maskell). This oviposition behavior has been considered a mechanism that affects defense reactions in other groups of insects (see Salt, 1968). Since encapsulation in a superparasitized host may not affect all the parasitoid eggs, one or more eggs can avoid encapsulation and develop normally. Therefore, superparasitism reduces the efficiency of encapsulation, although lowering the potential birth rate of the parasitoid. Increasing egg load (superparasitism) in an unsuitable host may be discovered as a common strategy of parasitoids to reduce the rates of effective encapsulation in mealybugs. Marked differences in the rates of EE between solitary and superparasitized mealybugs were evident in the nine combinations of the different ecotypes of *P. citri* and *A. pseudococci* that were studied. Likewise, it was found that multiple oviposition by *Apoanagyrus diversicornis* (Howard) in *Phenacoccus herreni* Cox and Williams clearly enhanced effective parasitism rates (Van Driesche *et. al.*, 1986). It was recently found that both *A. fusciventris* and *L. dactylopii* switch their egg distribution from uniform pattern as principal parasitoids to aggregative pattern as accidental parasitoids (Blumberg and Mendel unpublished). This typical behavior may evolve when a new host species is adapted.

The host-parasitoid combinations studied can be divided into four groups according to the levels of encapsulation and superparasitism. Mealybugs of group I display low values of both AE and EE, and low values of superparasitism. They are associated with a principal parasitoid and are considered most suitable for parasitoid development. Combinations included group II are characterized by high AE and EE values and by low rates of superparasitism. Combinations of group III are characterized by high AE and EE values and by high rates of superparasitism. Parasitoids involved in the second and third groups are accidental, and the mealybugs are considered unsuitable as hosts for their development. The level of superparasitism does apparently not affect EE values in these two groups. Group IV includes combinations of *P. citri* and *P. ficus* with *A. pseudococci*, which are characterized by high AE values, low EE values and high rates of superparasitism. We assume that these high rates of superparasitism constitute a strategy of the parasitoid to reduce EE values and thus enhance its survival. Both *Planococcus* spp. are most suitable for the development of *A. pseudococci* due to the low EE values, although *A. pseudococci* is probably not a principal parasitoid of the these two mealybugs.

Our findings also suggest that low rates of encapsulation are associated with mealybugs that are attacked by principal parasitoids and with similarity between the origin of the host and the parasitoid. High rates of encapsulation are probably associated with parasitism by accidental parasitoids and with different areas of origin of the host and the parasitoid. However, new association of parasitoid with host species closely related to its principal host may result in relatively low rates of encapsulation.

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