SUPERPARASITISM BY *TRICHOGRAMMA POLIAE* IN THE EGGS OF *CLOSTERA CUPREATA* (LEPIDOPTERA: NOTODONTIDAE) AND ITS EFFECT ON OFFSPRING

M. Ahmad*, M. J. Ahmad, R. K. Mishra & S. K. Sheel

Forest Entomology Division, Forest Research Institute, Dehra Dun - 248 006, Uttaranchal, India

Received August 1999

AHMAD, M., AHMAD, M. J., MISHRA, R. K. & SHEEL, S. K. 2002. Superparasitism by *Trichogramma poliae* in the eggs of *Clostera cupreate* (Lepidoptera: Notodontidae) and its effect on offspring. The paper describes the occurrence of "superparasitism" in the eggs of the poplar defoliator, *Clostera cupreata,* and in the laboratory host, *Corcyra cephalonica,* by the egg parasitoid, *Trichogramma poliae.* Superparasitism was determined by exposing host eggs and parasitoids in different numerical combinations and observing the number of offspring emerging from individual superparasitised eggs. The effect of superparasitism on the body size of emerged individuals was also determined. When the parasitoid:host ratio was 2:1, the incidence of superparasitism in the eggs of *C. cupreata* was 90% with an average number of 5.44 offspring developing per egg, and 40% in the eggs of *C. cephalonica,* with an average of 2.33 individuals per egg. Superparasitism declined markedly with increase in the relative availability of host eggs. Average body length of offspring obtained from superparasitised eggs of *C. cupreata* ranged from 0.34 to 0.39 mm for females and 0.25 to 0.27 mm for males in comparison with a mean body length from non- superparasitised eggs of 0.52 mm and 0.41 mm respectively. Similar reductions in size were observed in offspring from superparasitised eggs of *Corcyra cephalonica.* An inverse relationship between numerical emergence and body length of the parasitoid was also observed from superparasitised eggs of *C. cupreata.*

Key words: Superparasitism - *Trichogramma poliae* - *Clostera cupreata* - poplar - *Corcyra cephalonica* - eggs' volume - body length

AHMAD, M., AHMAD, M. J., MISHRA, R. K. & SHEEL, S. K. 2002. Superparasitisme oleh *Trichogramma poliae* di dalam telur *Clostera cupreata* (Lepidoptera: Notodontidae) dan kesannya terhadap anak peranggas. Artikel ini menerangkan mengenai kemunculan superparasitisme di dalam telur peranggas daun, *Clostèra cupreata,* dan di dalam perumah makmal, *Corcyra cephalonica* oleh parasitoid telur, *Trichogramma poliae.* Superparasitisme ditentukan dengan mendedahkan telur perumah dan parasitoid dalam kombinasi berangka yang berbeza dan mencerap bilangan anak yang muncul daripada telur yang disuperparasit. Kesan superparasitisme terhadap saiz badan individu yang muncul juga ditentukan. Apabila nisbah parasitoid:perumah ialah 2:1, kejadian superparatisme di dalam telur *C. cupreata* ialah 90% dengan purata 5.44 anak dihasilkan daripada setiap telur, dan 40% anak di dalam telur *C.cephalonica,* dengan purata 2.33 individu setiap telur. Superpasitisme merosot dengan ketara dengan pertambahan kemunculan secara relatif telur perumah. Purata panjang badan anak yang diperoleh daripada telur superparasitisme *C. cupreata* berjulat antara 0.34 hingga 0.39 mm bagi anak betina dan 0.25 hingga 0.27 mm bagi anak jantan berbanding min panjang badan daripada telur yang tidak disuperparasit iaitu masing-masing 0.52 mm

* *E-mail: ahmadm@icfre.up.nic.in*

dan 0.41 mm. Penurunan saiz yang serupa juga dicerap dalam anak daripada telur *C. cephalonia* yang disuperparasit. Perhubungan songsang antara kemunculan berangka dan panjang badan parasitoid juga dicerap dalam telur *C. cupreata* yang disuperparasit.

Introduction

The ability of parasitoids to recognise hosts that are already parasitised by conspecifics or by themselves is referred to as host discrimination. Parasitoids may either reject the host already parasitised or lay a second clutch of eggs in them. The latter is called "superparasitism".

The term was originally defined as super abundance of parasites of a single species attacking an individual host (Smith 1916). Various explanations concerning the circumstances which encourage the occurrence of superparasitism have been offered. Some regard the phenomenon of superparasitism as a behavior of the parasitoids that is expressed when unattacked hosts are relatively rare (Roitberg & Mangel 1988, Papaj *et al.* 1989). According to this interpretation a female parasite oviposits in an already parasitised host because of non-availability of unparasitised hosts in sufficient number for all of her eggs (Parker & Courtney 1984, Mangel & Clark 1988). Most superparasitism cases have been described in support of this interpretation (Hubbard *et al.* 1987, Bai & Mackauer 1990, Visser *et al.* 1990, Speirs *et al.* 1991, Roitberg *et al.* 1992, van Alphen *et al.* 1992). In all probability, superparasitism occurs under either one of two possible situations; firstly, when parasitoids fail to discriminate an already parasitised host and secondly, under stressed conditions when unparasitised hosts are unavailable. In either of these situations, superparasitism affects subsequent parasitoid development.

Trichogramma poliae is one of the five egg parasitoids which has been found indigenously associated with the eggs of poplar defoliator, *Clostera cupreata*, in the Tarai regions of Uttaranchal and Haryana states of India (Ahmad *et al.* 1997, 1999). *Clostera cupreata* is a prime defoliator of poplar (*Populus deltoides*) and often causes epidemic defoliation in main plantation areas of northern India (Seth 1969, Lohani 1976, Chaturvedi 1979, Singh *et al.* 1983). The phenomenon of superparasitism by *T. poliae* was observed in the eggs of *C. cupreata* collected from the field. The present study was undertaken to understand the occurrence and extent of superparasitism by *T. poliae* under different conditions in the eggs of its natural host, *C. cupreata* and the consequent effects of superparasitism on the offspring. The phenomenon of superparasitism was also studied in the eggs of *Corcyra cephalonica* (Lepidoptera: Pyralidae) which has been used as laboratory host to this parasitoid.

Materials and methods

A culture of *Clostera cupreata* was maintained in wooden glass cages measuring 50 × 65 × 50 cm by feeding the larvae on poplar foliage. The egg parasitoid, *T. poliae*, was bred on the eggs of the laboratory host, *Corcyra cephalonica*, in culture tubes measuring 150 × 25 mm. Egg cards of *C. cephalonica* were prepared in the manner described by Ahmad *et al.* (1999). To obtain eggs of *C. cupreata* for experimental purpose, freshly emerged female and male moths were released into a rearing jar, with its inside wall lined with white paper. The mouth of the jar was also covered with white paper, held with rubber band. Diluted honey solution soaked in cotton ball was provided as food inside the jar. The eggs were deposited on the white paper which was collected after 24 hours. The paper was cut into small strips containing one, two and five eggs and pasted on egg cards measuring 75 × 20 mm. Only viable eggs indicated through appearance of bands were considered for use. Aspects on superparasitism were studied by taking five different treatments of parasitoids:host egg ratio, namely, 1:1, 2:1, 5:1, 1:2 and 1:5, for both natural as well as laboratory hosts. A 'control' condition (denoted by F1 and M1) was maintained for comparison, where more than 100 eggs were exposed to a pair of parasitoids. Egg cards with definite number of host eggs were exposed to one, two and five pairs of freshly emerged *T. poliae* for 48 hours in individual tubes plugged at the mouth with cotton balls. The eggs were then separated and observed for the emergence of female and male parasitoids. Normally emergence of parasitoids occured nine days after parasitisation and is completed within two to three days. After completion of emergence remaining unemerged eggs were dissected to determine the number of undeveloped parasitoids. Similar experiments were conducted using the eggs of the laboratory host, *C. cephalonica*, where emergence of parasitoids started after eight days of parasitisation and completed within one or two days.

Total number of parasitoids emerging from the eggs of both the natural as well as laboratory hosts as a result of each treatment was counted and emergence of average number of individuals per egg was calculated. Percentage of superparasitism in different treatments was also determined.

The effect of superparasitism on the size of emerging parasitoids was assessed by measuring the total body length, from vertex to the tip of abdomen of emerged parasitoids. A total of 25 individuals in each treatment was measured for this purpose. F2–F6 and M2–M6 (Table 1) indicate body length of superparasitised females and males respectively, corresponding to five different treatments of parasitoid:host egg ratio. F1 and M1 indicate body length of respective normal female and male. The total capacity (volume) of host eggs available to parasitoids was determined using formulae appropriate to the different shape of the eggs:

(a) hemispherical (*C. cupreata*)

$$2/3 \pi r^3$$

where

r = radius of the egg

(b) elliptical (*C. cephalonica*)

$$4/3 \pi r_1 * r_2 * r_3$$

where

r_1, r_2 and r_3 indicate radii of length, width and height of the egg respectively

Stereoscopic binocular (Leica-Wild M10) with oculometer arrangement was used to take body measurements as well as dimensions of host eggs. All experiments were conducted at a temperature of 27 ± 1°C and 65 ± 5% relative humidity and were replicated ten times.

Table 1 Average body length of superparasitised female and male of *Trichogramma poliae* and level of significance of variations

Character	Source of variation	Bar diagram on mean values						CD	Significance level
Body length by *Clostera* eggs	Females	F1 0.523	F2 0.390	F3 0.378	F4 0.360	F5 0.358	F6 0.344	0.038	***
	Males	M1 0.407	M2 0.266	M3 0.256	M4 0.254	M5 0.254	M6 0.254	0.031	***
Body length by *Corcyra* eggs	Females	F1 0.437	F2 0.344	F3 0.344	F4 0.343	F5 0.341	F6 0.333	0.035	***
	Males	M1 0.332	M2 0.253	M3 0.250	M4 0.250	M5 0.249	M6 0.233	0.031	***
Body length of females	Types of eggs		*Clostera* 0.3928	>	*Corcya* 0.3569			0.008	***
Body length of males	Types of eggs		*Clostera* 0.2819	>	*Corcya* 0.2614			0.007	***
Body length	Sex		Female 0.374	>	Male 0.274				
Body length ratio (F/M) from *Clostera* eggs		R3 1.52	R2 1.49	R5 1.45	R4 1.44	R6 1.38	R1 1.31	–	ns
Body length ratio (F/M) from *Corcyra* eggs		R2 1.50	R4 1.39	R5 1.39	R6 1.38	R3 1.35	R1 1.35	0.035	***

F = female, M = male, R = ratio

Statistical analysis

The data on body length of female and male obtained from the host eggs, *C. cupreata* and *C. cephalonica,* under different treatments were analysed by one-way ANOVA to ascertain the extent of variation among the body length. Variation in the body length of superparasitised females (F2–F6) and males (M2–M6) was tested for significance and compared with those of non-superparasitised females (F1) and males (M1) at probability level of 0.1%. Average body-length of superparasitised females and males obtained from two different host eggs were also compared using ANOVA of two-factor with replication. Regression equation was also fitted for predicting inverse relationship between body length and number of *T. poliae* emerged/egg of *C. cupreata,* after transforming the values into logarithmic scale. Female to male body length ratio in each case was also worked out to observe any relationship that resulted from the various parasitoid:host egg ratio. Student's *t*-test was applied to test the level of significance on the numerical emergence of parasitoids per egg from two different hosts, as a result of different treatments.

Results

Superparasitism by *T. poliae* was observed in both the natural as well as laboratory host eggs. However, it was more prevalent in the natural host with a maximum 90% incidence compared with 10 to 40% in the eggs of *Corcyra cephalonica* (Figure 1). There were only marginal differences in the occurrence of superparasitism when different numbers of parasitoids were offered a single host egg. A parasitoid:host ratio of 1:1 resulted in 80% superparasitism of *Clostera cupreata* eggs. This increased to 90% at parasitoid:host ratio of 2:1 and 5:1. In the case of *C. cephalonica* 30% superparasitism was observed at a ratio of 1:1 increasing to 40% at ratios of 2:1 and 5:1. Although the incidence of superparasitism was greater at lower levels of host availability in these experiments, the mean number of parasitoids emerging successfully, however, did not increase significantly. This was because of over-infestation of eggs, which subsequently suffered desiccation at an early stage. This resulted in insufficient food to support the growing larvae, resulting in their premature death. Experiments conducted by exposing single pairs of parasitoids to two and five host eggs of each species increased the relative availability of host eggs and substantially lowered the rate of superparasitism. At a 1:2 parasitoid:host ratio 70% superparasitism was observed in the eggs of *C. cupreata.* This further decreased to only 40% at a parasitoid:host ratio of 1:5. Similarly, the rate of superparasitism decreased to 30 and 10% respectively in the case of *C. cephalonica* eggs (Figure 1).

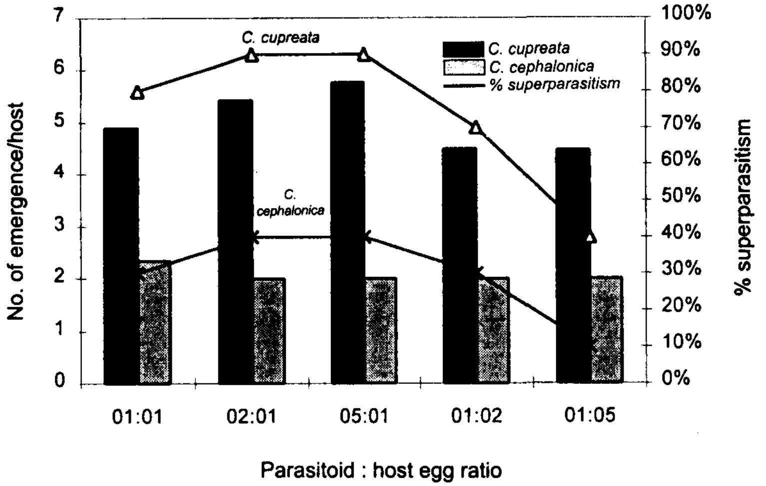


Figure 1 Numerical emergence of *Trichogramma poliae* at varying parasitoid:host egg ratio

There was a considerable difference in the mean number of parasitoids which successfully emerged from a single egg of each host species. As many as nine parasitoids per egg were observed from *C. cupreata* with a mean of 5.44 ± 1.00 per egg at a parasitoid:host ratio of 2:1. When *C. cephalonica* was used as the host, a maximum of three individuals per egg was observed with a mean of 2.25 ± 0.50 at a parasitoid:host ratio of 1:1 (Figure 1). The difference in numerical emergence was statistically significant at 0. 1% level of probability when compared using *t*- test.

The observed number of parasitoids emerging from each host species was clearly related to the size and potential capacity of the eggs of each host. However the number of successful parasitoids did not reach the potential capacity of each host's eggs based on a simple calculation of egg volume. A maximum of nine parasitoids per egg was observed from *C. cupreata* while the estimated volumetric capacity (0.073 mm^3) of their host egg was 14.6 parasitoids per host egg based on a calculated requirement of 0.005 mm^3 per parasite (Table 2). Similarly, the average volume of *C. cephalonica* eggs (0.024 mm^3) provided a theoretical capacity of 4.83 parasitoids per egg but emergence was limited to a maximum of three parasitoids per egg.

Table 2 Potential and observed maximal capacities of natural and laboratory host eggs for *Trichogramma poliae*

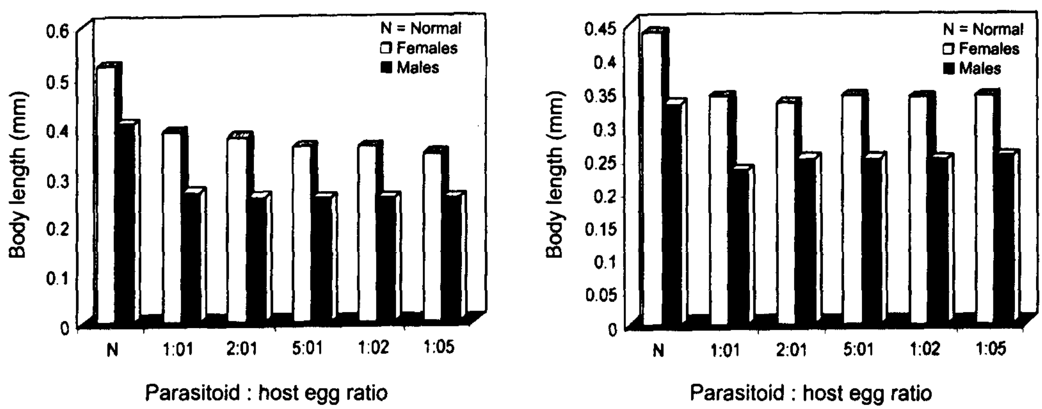
Host eggs	Mean volume of egg (mm^3)	Estimated volume requirement/ *T. poliae* (mm^3)	Estimated potential parasitoid capacity/ host egg	Maximum observed no. of parasitoids/ host egg
Clostera cupreata	0.073	0.005	14.60	9
Corcyra cephalonica	0.024	0.005	4.83	3

The body length of parasitoids resulting from superparasitism was comparatively less than normal (Figure 2). In the case of *C. cupreata* the average body length of female parasitoids varied from 0.34 to 0.39 mm in superparasitism cohorts compared to 0.52 mm for normal females. Similarly the body length of male parasitoids varied from 0.25 to 0.26 mm compared with a normal male length of 0.41 mm (Figure 2(a)). Considerably greater variation in size was observed among individuals resulting from superparasitism. Some females from the natural host were as small as 0.21 mm while others measured 0.47 mm, exceeding the minimum size (0.42 mm) of normal females. Similarly, the size of males ranged up to 0.37 mm which is larger than minimum length (0.33 mm) of normal males. Decline in the body length of superparasitised cohorts was found to be strongly related to the number of individuals emerging per host egg. This relationship was more prominent in the case of superparasitised eggs of *C. cupreata* where body length of parasitoids was found to be inversely proportional to the number of individuals resulting from the same host egg (Figure 3). Regression analysis based on data on the body length and number of individuals emerged/host egg yielded the following predictive equation:

$$\log y = -0.5896 + 0.6189\,x \quad (r^2 = 0.9996)$$

where

x = number of individuals emerged/host egg,
y = body length of parasitoids and
r^2 = coefficient of determination.



(a) *Clostera cupreata* (b) *Corcyra cephalonica*

Figure 2 Variation in body length of superparasitised *Trichogramma poliae* at varying parasitoid: host ratio

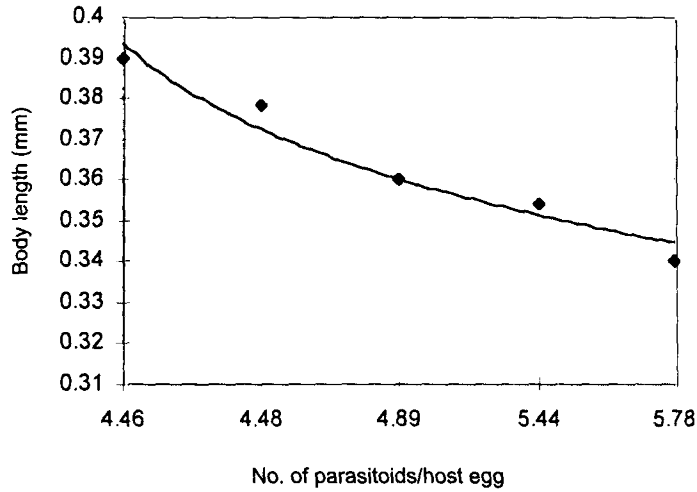


Figure 3 Relationship between numerical emergence and body length of *Trichogramma poliae* from *Clostera cupreata*

The value of r^2 indicated strong predictability of inverse relationship between x and y. Although the body length declined with the rise in numerical emergence of parasitoids per host egg, it stabilised after a certain value. As the variation in data on similar parameter from superparasitised eggs of *C. cephalonica* was less pronounced, regression analysis showed no significant relationship between body length and number of parasitoids emerged per host egg. Average body length of superparasitised females from the eggs of *C. cephalonica* in different treatments varied from 0.33 to 0.34 mm with minimum and maximum value being 0.24 to 0.43 mm respectively. Average body length for males varied from 0.23 to 0.25 mm in different treatments while extreme size ranged from 0.19 to 0.33 mm (Figure 2(b)). Average body length of normal female parasitoids from *C. cephalonica* eggs was 0.4 mm (0.24 to 0.43 mm) while that of normal males 0.33 mm (0.19 to 0.33 mm). In some cases, the maximum size attained by the superparasitised forms was close to the average size of normal individuals but none reached the maximum length of normal individuals.

One-way ANOVA analysis showed that variation among body length of superparasitised females and males obtained from the eggs of natural and laboratory hosts as a result of different treatments was statistically insignificant at probability level of 0.1%. The values, however, were found to be significantly different at the probability level of 0.1% when compared with normal body length of female and male (Table 1). Body length of parasitoids from natural host was always greater than those emerged from the eggs of laboratory host. Similar observation was made in the superparasitised forms. Comparison of samples of superparasitised females of the two different hosts revealed a significant difference in body length when analysed through ANOVA two- factor with replication (d.f. = 249, p = 0.001, F = 15.466, Fcnt = 3.88). However insignificant variation in body length was observed in superparasitised males (d.f. = 249, p = 0.001, F = 3.279, Fcnt = 3.88). In each case females were found to be larger than males but body length ratio of female: male as obtained from different parasitoid:host egg ratio was nearly the same, varying insignificantly at the 0.1% probability level.

Discussion

Although the incidence and extent of superparasitism in natural condition is yet to be explored, it was however found to be as high as 90% in *Clostera cupreata* and up to 40% in *Corcyra cephalonica*. Rate of superparasitism was considerably minimised when one pair of parasitoids was exposed to increased number of host eggs. This might be due to the availability of healthy eggs within reach and ability to recognise already parasitised eggs. Display of superparasitism under the given parasitoid:host egg ratio alludes exploitation of an individual egg by female parasitoid under specific circumstances. The egg- laying cavity made by the female for first oviposition are reused for depositing multiple eggs in order to save time (Papaj & Pimontel 1997) and effort for making fresh hole puncturing the relatively hard outer covering of the egg.

Significant difference in the observed data on the numerical development of parasitoids, when compared with calculated capacity of eggs, reflected involvement of some regulatory control over the extent of superparasitism. Extra space inside an eggs is resulted from the interspaces between growing individuals, which is essential for proper development of parasitoids.

An overall decrease in the body length of superparasitised offsprings of *T. poliae* emerging from host eggs indicated availability of limited space in the host egg for the development of either one parasitoid (normal condition) or more (superparasitised). Similar logic can be used to explain the inverse relationship between body length and number of *T. poliae* that emerged from a single superparasitised egg of *C. cupreata*. Relatively smaller-sized offspring of *T. poliae* emerging from eggs of *C. cephalonica* as compared to *C. cupreata* indicated space dependent development of parasitoids. Presence of superparasitism is advantageous for the survival of parasitoid in the event of limited availability of host eggs.

Acknowledgements

The present study is a part of FREE Project ICFRE - 6, financed by World Bank. The authors express sincere thanks to J. K. Rawat, Director, Forest Research Institute, Dehra Dun for his kind help and encouragement.

References

AHMAD, M., AHMAD, M. J. & MISHRA, R. K. 1997. Indigenous microhymenopteran parasitoid species associated with poplar defoliator, *Clostera cupreata* (Lepidoptera: Notodontidae). *Annals of Forestry* 5(1): 26–34.

AHMAD, M., AHMAD, M. J. & MISHRA, R. K. 1999. *Trichogramma poliae* Nagaraja (Hymenoptera: Trichogrammatidae): a new record on poplar defoliator, *Clostera cupreata* (Lepidoptera: Notodontidae) from India: its population dynamics and bioefficacy. *Annals of Forestry* 7(1): 9–19.

BAI, B. & MACKAUER, M. 1990. Host discrimination by the aphid parasitoid *Aphelinus asychis* (Hymenoptera: Aphelinidae): when superparasitism is not adaptive. *Canadian Entomology* 122: 363– 372.

HUBBARD, S. F., MARRIS, G., REYNOLDS, A. & ROWE, G. W. 1987. Adaptive patterns in the avoidance of superparasitism by solitary parasitic wasps. *Journal of Animal Ecology* 56: 387– 401.

MANGEL, M. S. & CLARK, C. W. 1988. *Dynamic Modelling in Behavioural Ecology.* Princeton University Press, Princeton. 320 pp.

PAPAJ, D. R. & PIMONTEL, A. H. 1997. Why walnut flies superparasitise: time saving as a possible explanation. *Oecologia* 109(1): 166–174.

PAPAJ, D. R., ROITBERG, B. D. & OPP, S. B. 1989. Serial effects of host infestation on egg allocation by the Mediterranean fruit fly: a rule of thumb and its functional significance. *Journal of Animal Ecology* 58: 955–970.

PARKER, G. A. & COURTNEY, S. P. 1984. Models of clutch size in insect oviposition. *Theoretical Population Biology* 26: 27–48.

ROITBERG, B. D. & MANGEL, M. S. 1988. On the evolutionary ecology of marking pheromones. *Evolution and Ecology* 2: 289–315.

ROITBERG, B. D., MANGEL, M. S., LALONDE, R. G., ROITBERG, C. A., VAN AALPHEN, J. J. M. & VET, L. 1992. Seasonal dynamic shifts in patch exploitation by parasitic wasps. *Behavioural Ecology* 3: 156–265.

SINGH, P., RAWAT, D. S., MISRA, R. M., PRASAD, G. & TYAGI, B. D. S. 1983. Epidemic defoliation of poplars and its control in Tarai Central Forest Division, Uttar Pradesh. *Indian Forester* 109(9): 675– 693.

SMITH, H. S. 1916. An attempt to redefine the host relationship exhibited by entomophagous insects. *Journal of Economic Entomology* 9: 477– 486.

SPEIRS, D. G., SHERRATT, T. N. & HUBBARD, S. F. 1991. Parasitoids diets: does superparasitism pay? *Trends in Ecology and Evolution* 6: 22–25.

VAN ALPHEN, J. J. M., VISSER, M. E. & NELL, H. W. 1992. Adaptive superparasitism and patch time allocation in solitary parasitoids: searching in groups vs. sequential patch visits. *Functional Ecology* 6: 528–535.

VISSER, M. E., VAN ALPHEN, J. J. M. & NELL, H. W. 1990. Adaptive superparasitism in patch time allocation in solitary parasitoids: the influence of the number of parasitoids depleting the patch. *Behaviour* 114: 21–36.