Variation in Total and Differential Haemocyte Count of *Busseola fusca* (Lepidoptera: Noctuidae) Parasitized by Two Biotypes of *Cotesia sesamiae* (Hymenoptera: Braconidae) and Larval Growth Responses

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ABSTRACT *Cotesia sesamiae* (Cameron) is an indigenous larval endoparasitoid that attacks mid- to late-stage gramineous stem borer larvae in Africa. Two biotypes of *C. sesamiae* have been reported with differential abilities to suppress the immune system of *Busseola fusca* Fuller. Eggs of a *C. sesamiae* population from Mombasa were encapsulated, whereas eggs of a population from Kitale were not. Total and differential hemocytes were counted in larvae of *B. fusca* at six times (2 h, 14 h, 24 h, 72 h, 120 h, 168 h) after being exposed to parasitoids from Kitale and Mombasa. The total numbers of hemocytes in a larva parasitized by the *C. sesamiae* population from Mombasa were higher as compared with larvae parasitized by the *C. sesamiae* population from Kitale. Plasmatocytes, in particular, were reduced in larvae parasitized by *C. sesamiae* from western Kenya from 72 to 168 h after oviposition. Our results suggest that plasmatocytes probably play an important role in the immune response of *B. fusca.* Significant proportions of the host larvae were still at the larval stage for those parasitized by *C. sesamiae* from Mombasa and another species of *Cotesia*, *Cotesia flavipes* Cameron at day 12. A reduction of *B. fusca* larval weight was observed on day 12 after oviposition by *C. sesamiae* from Kitale.

KEY WORDS *Cotesia sesamiae*, hemocytes, parasitism, plasmatocytes, granulocytes

FOR SUCCESSFUL DEVELOPMENT of endoparasitoids, the immune system of their hosts must be evaded, and much research has been conducted on the mechanisms by which parasitoids evade immune systems (Osman 1978, Rizki and Rizki 1984, Vinson 1993, Strand et al. 1997). In insects, the immune system includes humoral and cellular components. The cellular component involves a number of single free-floating cells in the hemolymph, collectively referred to as hemocytes (Vinson 1993). A key factor to the successful development of endoparasitoids is their ability to suppress hemocytic activity, which is characterized by a decline in the responsiveness to the evasion of parasitoid eggs.

Encapsulation, and in some cases nodule formation, is a common response of host insects to invading foreign organisms and inert materials (Vinson 1990). In most Lepidoptera, granular cells and plasmatocytes are the key hemocytes involved in encapsulation (Strand and Pech 1995). The mechanism of encapsulation has been described by Vinson (1990) and Strand and Pech (1995). Initially, granular hemocytes recognize the foreign target as "nonself" and adhere to its surface. The attached granular hemocytes degenerate and release factors assumed to attract plasmatocytes. The degenerating granular hemocytes are also presumed to release factors that coat the foreign object and allow the plasmatocytes to attach. Plasmatocytes then become mutually adhesive and form a multicellular sheath around the target. Studies with *Galleria mellonella* (L.) and *Clitumnus extradentatus* (Brunner) (Ratcliffe and Gagen 1997; Schmit and Ratcliffe 1977, 1978) showed that granular cells attached and lysed on the surface of the target, and released material that recruited and/or mediated the attachment of plasmatocytes. In a recent study, Wiegand et al. (2000) reported a monoclonal antibody against *Manduca sexta* (L.) plasmatocytes that effectively inhibited encapsulation of synthetic beads in vitro and in vivo, and they effectively inhibited plasmatocytes spreading in vitro. Prevost et al. (1990) reported on the effects of parasitism on the total and differential hemocyte counts. In resistant larvae, a decreased number of granulocytes and an increased circulation of plasmatocytes were observed. Several authors have identified the involvement of plasmatocytes in capsule formation in various insects (Ratcliffe and Rowley 1979, Gotz 1986, Ratcliffe 1986). In most lepidopteran and dipteran hosts, successful parasitization led to a reduction in the total number of hemocytes in circulation (Davies et al. 1987, Guzo and Stoltz 1987, Prevost et al. 1990).

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Cotesia sesamiae (Cameron) is an indigenous, gregarious, larval endoparasitoid that attacks mid- to late-stage stem borer (Lepidoptera: Crambidae, Noctuidae) larvae in the field including the native species *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and the exotic pest *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). *C. sesamiae* is reported to be one of the most important native larval parasitoids of maize and sorghum stem borers in many countries in sub-Saharan Africa (Ingram 1958, Mohyuddin 1971, Scheibelreiter 1980, Polaszek and Walker 1991, Walker 1994, Overholt et al. 1994a, Kfir 1995).

In eastern and southern Africa, a classical biological control program has been started in an attempt to reduce *Chilo partellus* populations. In this program, an exotic parasitoid, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was introduced into Kenya for the control of *Chilo partellus* and established (Omwega et al. 1997). Previous studies have shown that *C. flavipes* would inject its eggs in *B. fusca* when offered for oviposition (Ngi-Song et al. 1996, Ngi-Song and Overholt 1997), but all eggs were encapsulated (Ngi-Song et al. 1995, 1998). A recent study of multiple parasitism by *C. sesamiae* and *C. flavipes* on *B. fusca* by Ngi-Song et al. (2001) demonstrated that when the larvae were parasitized by *C. sesamiae* first, and then 2 h later by *C. flavipes*, and vice versa, most of the progeny were *C. flavipes.* However, when *B. fusca* larvae were stung by *C. sesamiae* 3 d before oviposition by *C. flavipes*, significantly more *C. sesamiae* emerged from the larvae. When *C. flavipes* oviposited first, no host larvae produced only *C. flavipes.*

The survival of insect endoparasitoids depends on multiple factors including biochemical and hormonal interaction and on suppression of the host's immune system. Suppression is modulated by complex mechanisms to help the parasitoid develop successfully (Lavine and Beckage 1995). Polydnaviruses that are injected by the female parasitoid in the host hemocoel during oviposition (Lavine and Beckage 1995) have been shown to cause most of the physiological effects in the host, such as changes in growth, development, behavior, and hemocytic activity (Davies et al. 1987, Vinson et al.1979, Vinson and Stoltz 1986). A recent study by Mochiah et al. (2002) showed that injecting calyx fluid of *C. sesamiae* from western Kenya allowed the coastal form to develop in *B. fusca.*

In previous studies, Ngi-Song et al. (1998) suggested that two geographical populations of *C. sesamiae* existed with differential abilities to suppress the immune system of *B. fusca*, which is a native pest. *C. sesamiae* from the coastal area of Kenya was not able to develop in *B. fusca* because all eggs were encapsulated. However, *C. sesamiae* from western Kenya successfully developed in *B. fusca.* Here we report on the results of a study that evaluated the effect of parasitism by these two populations of *C. sesamiae* on the total and differential hemocyte counts of *B. fusca.* This study also evaluated the effects of parasitism by two biotypes of *C. sesamiae* and *C. flavipes* on growth and development of *B. fusca* larvae.

Materials and Methods

Parasitoids. A colony of *C. flavipes* that originated from Pakistan was maintained on laboratory reared *Chilo partellus* larvae according to methods described by Overholt et al. (1994b). *C. sesamiae* were collected in a survey conducted in Kenya during the long (April–June) and short (September–November) rains in 1998 from Kitale in western Kenya and Mombasa on Kenya's southeast coast. Parasitoids from Kitale were collected from *B. fusca*, and the coastal material was collected from *Chilo partellus.* The identification of the *C. sesamiae* was confirmed by examining the male genitalia (Kimani-Njogu and Overholt 1997). Voucher specimens have been deposited in the collections of the Biosystematic Unit at the International Centre of Insect Physiology and Ecology (ICIPE).

Laboratory colonies of these two populations of *C. sesamiae* were initiated and reared by a hand-stinging method described previously by Overholt et al. (1994b). For standardization, each parasitoid population was reared on *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), a host suitable for the development of both populations (Ngi-Song et al. 1995), for more than 10 generations before the experiments were initiated. After parasitization, hosts were maintained on artificial diet at 25°C, 50–65% RH, and 12L: 12D h photoperiod. Parasitoid cocoons were collected in glass vials (2.5 × 7 cm) and kept in a clean perpex cage until emergence, after which adult parasitoids were fed a 20% honey–water solution.

Hosts. A colony of *B. fusca* was initiated with materials collected from western Kenya, and the *S. calamistis* colony was initiated with insects collected in the southern coastal area of Kenya. Larvae were reared on artificial diet (Onyango and Ochieng-Odero 1994).

Total and Differential Hemocyte Counts. Total (THC) and differential hemocyte counts (DHC) were made on individual fourth-instar *B. fusca* using the method of Davies et al. (1987). Larvae of approximately the same size were used. Larvae were assayed at six times (2 h, 14 h, 24 h, 72 h, 120 h, 168 h) after oviposition by parasitoids from the two populations. A cohort of 180 of fourth-instar *B. fusca* was exposed to each parasitoid population. Care was taken to allow only one oviposition per host.

After oviposition, the larvae were transferred to glass vials (2.5 × 7 cm) containing diet and kept in an incubator at 25°C. An equal number of unparasitized larvae were maintained as a control. At each assay time, five larvae from each treatment were randomly selected. Each test larva was heated in an oven at 60°C for 1–2 min to prevent coagulation of hemolymph and to immobilize the larvae. A proleg was cut, and hemolymph was quickly squeezed onto a sheet of Parafilm. These experiments were carried out simultaneously on the same cohort of hosts to be sure that the differences observed between the values of THC and DHC were not variations caused by time of collection of hemolymph. Aliquots of 4 μl of hemolymph were diluted in 36 μl of a saline solution in which an

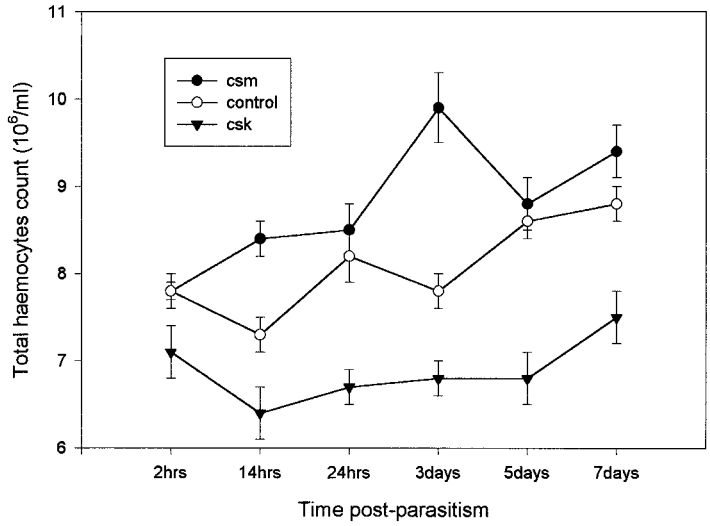


Fig. 1. Total hemocyte counts of *B. fusca* parasitized by CSM, *C. sesamiae* from Mombasa; CSK, *C. sesamiae* from Kitale, and unparasitized *B. fusca* (control).

anticoagulant (0.15 M NaCl, 5 mM KPO_4, citric acid pH 6.5) had been added to prepare a 1:10 dilution. Aliquots of 10 μl of this sample were then transferred immediately to an improved Neubauer hemocytometer (Cole Parmer, Chicago). Two hemocyte types, plasmatocytes and granulocytes, were distinguished using the classification scheme of Rowley and Ratcliffe (1981). Total hemocytes were counted from 15 grids using a compound microscope. This test was replicated six times with five larvae per replicate for each of the treatments and times after oviposition. Differential hemocytes were also counted and expressed as the relative number of different types of hemocytes per total number of hemocytes counted at a given time.

Encapsulation Experiment. Cohorts of 20 fourth-instar *B. fusca* were parasitized by the two populations of *C. sesamiae.* Ten of the parasitized host larvae from Mombasa were dissected 4–5 d after oviposition to check for encapsulation. The remaining host larvae and those parasitized from Kitale were inspected daily for pupation or cocoon formation.

Larval Growth and Development Experiment. To establish whether parasitization affected larval growth and development, we weighed *B. fusca* fourth instars before offering them for parasitization. *B. fusca* larvae were exposed to the two biotypes of *C. sesamiae* and *C. flavipes.* There were 51 replicates for each parasitoid population. A control consisted of the same number of unparasitized larvae. Parasitized larvae were reared on artificial diet (Onyango and Ochieng-Odero 1994) in an incubator at 25°C ± 1, 60–70% RH, and 12L:12D h. Larvae were inspected and weighed again after 1, 3, 5, 7, 9, and 12 d. Mortality, pupation, and parasitoid emergence were recorded at each weighing.

Data Analysis. The percentages of each hemocyte type were transformed to cells/ml using the mean total hemocyte count. Percentage granulocytes and plasmatocytes counts were log transformed before being analyzed using a general linear model procedure. When ANOVAs (analysis of variance) were significant ($P < 0.05$), means were separated using Tukey's test. A two-way interaction between population and time was also applied to counts of each of the cell types recorded. Larval weights were analyzed by one-way ANOVA, and treatment means were compared against control means by Tukey's procedure. Pupation, larval stage, mortality, and cocoon formation were compared after day 12 by a chi-square test. All analyses were performed using the SAS program (SAS Institute 1999–2000).

Results

Parasitisation of *B. fusca* by the *C. sesamiae* population from Mombasa caused a significant increase in the THC, but the THC was greatly reduced in larvae parasitized by the *C. sesamiae* from Kitale (Fig. 1). The THC of larvae parasitized by the *C. sesamiae* population from Mombasa and those from the control population were observed to be significantly higher than counts from larvae parasitized by the *C. sesamiae* population from Kitale at all the sampling times (2 h: $F = 4.70$, df = 2, 87, $P = 0.0115$; 14 h: $F = 39.94$, df = 2, 87, $P = 0.0001$; 24 h: $F = 15.48$, df = 2, 87, $P = 0.0001$; 72 h: $F = 12.73$, df = 2, 87, $P = 0.0001$; 120 h: $F = 15.45$, df = 2, 87, $P = 0.0001$; 168 h: $F = 8.87$, df = 2, 87, $P = 0.003$). The maximum count of 9.9×10^6 cell/ml was observed at 72 h after oviposition by *C. sesamiae* from Mombasa, with a decline at 120 h followed by an increase on the 168 h of sampling (Fig. 1). The interaction between time and population in the THC was found to be significant (Table 1).

Approximately 70–80% of total hemocytes were plasmatocytes and granulocytes. Other hemocytes ob-

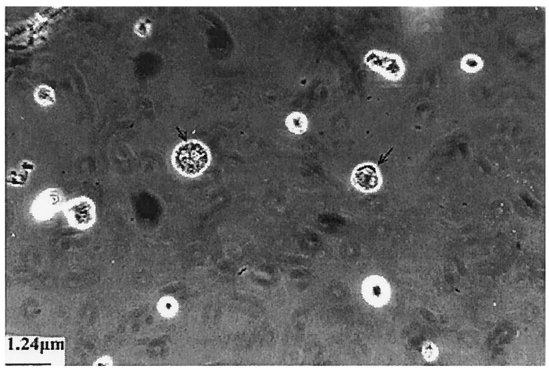
Table 1. The effect of time after oviposition, *C. sesamiae* population, and the interaction of time and population on the proportion of plasmatocytes and granulocytes in *B. fusca* larvae

Factor	THC[a]			Plasmatocytes			Granulocytes		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
Time	9.50	5, 517	0.0001	11.41	5, 517	0.0001	1.94	5, 517	0.126
Population	89.41	2, 517	0.0001	35.71	2, 517	0.0001	9.00	2, 517	0.0007
Time × population	3.70	10, 517	0.0001	3.16	10, 517	0.0006	2.10	10, 517	0.0227

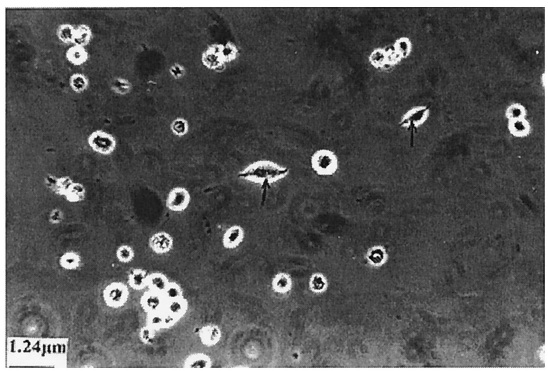
Analysis of variance was performed by GLM, SAS Institute. Significance level at $P < 0.05$.
[a] THC, total haemocyte counts

served were spherule cells, oenocytoids, or prohaemocytes but they are not discussed in this study. Granulocytes were spherical, contained more granules than the plasmatocytes and accounted for 45–50% of total hemocytes (Fig. 2A). Plasmatocytes, however, were mostly spindle-shaped, contained fewer granules than the granulocytes and accounted for between 20 and 25% of total hemocytes (Fig. 2B).

The percentage of plasmatocytes to THC at 2 h was significantly higher in host larvae stung by *C. sesamiae* from Mombasa as compared with counts taken from larvae parasitized by *C. sesamiae* from Kitale or the control ($F = 4.1$, df = 2, 87, $P = 0.0199$). No significant difference was observed between the two treatments and the control in plasmatocyte counts at 14 and 24 h ($F = 0.24$, df = 2, 87, $P = 0.7887$; $F = 1.49$, df = 2, 87, $P = 0.2307$). However, at 72 h to 168 h, the percentage of plasmatocytes was significantly higher in *B. fusca* larvae parasitized by *C. sesamiae* from Mombasa compared with larvae parasitized by the Kitale population (72 h: $F = 33.62$, df = 2, 87, $P = 0.0001$; 120 h: $F = 4.1$, df = 2, 87, $P = 0.0001$; 168 h: $F = 4.1$, df = 2, 87, $P = 0.0001$) (Fig. 3). Significant interaction was observed between population and time in the percentage of plasmatocytes (Table 1). Similarly, percentage of granulocytes showed no significant difference from 2 h to 72 h among the three populations (2 h: $F = 0.38.1$, df = 2, 87, $P = 0.6858$; 14 h: $F = 0.69$, df = 2, 87, $P = 0.5023$; 24 h: $F = 0.47$ df = 2, 87, $P = 0.6247$; 72 h: $F = 1.42$, df = 2, 87, $P = 0.2478$). However, at 120 and 168 h, the percentages of granulocytes were significantly higher in larvae stung by *C. sesamiae* from Mombasa and *C. sesamiae* from Kitale, as compared with the control ($F = 10.17$, df = 2, 87, $P = 0.0001$; $F = 4.74$ df = 2, 87, $P = 0.0111$) (Fig. 3). A significant interaction was also observed between populations and time after oviposition in the percentage of granulocytes (Table 1).



(A)



(B)

Fig. 2. Phase contrast micrographs of (A) granulocytes (arrows), (B) spindle-shaped plasmatocytes (arrows) from *B. fusca* larvae parasitized 24 h earlier by *C. sesamiae* from Mombasa.

No significant difference was observed between the two treatments and the control in the variation of plasmatocytes and granulocytes in relation to the type of cells (spherules, oenocytoids, and prohaemocytes) from 2 h to 24 h ($F = 0.10$, df = 2, 87, $P = 0.9018$; $F = 0.02$ df = 2, 87, $P = 0.9758$; $F = 0.43$, df = 2, 87, $P = 0.6500$. However, at 72 h to 168 h, the variation of plasmatocytes and granulocytes in relation to the other category of cells was significant in *B. fusca* larvae parasitized by *C. sesamiae* from Mombasa, Kitale, and the control ($F = 5.47$, df = 2, 87, $P = 0.0058$; $F = 4.74$ df = 2, 87, $P = 0.0105$; $F = 3.16$, df = 2, 87, $P = 0.0402$) (Fig. 3).

Dissection of the larvae parasitized by the *C. sesamiae* population from Mombasa revealed a capsule of hemocytes around the eggs (Fig. 4) and arrested development of the parasitoid. However, in the larvae parasitized by the Kitale population, parasitoid eggs hatched, and larvae developed to maturity and spun cocoons.

Larval Growth and Developmental Responses. The average weights of larvae parasitized by the two biotypes of *C. sesamiae* and *C. flavipes* did not differ from day 1 to day 5 compared with the control. However, the average weight of larvae parasitized by *C. sesamiae* from Kitale was higher at days 7 and 9 but reduced at

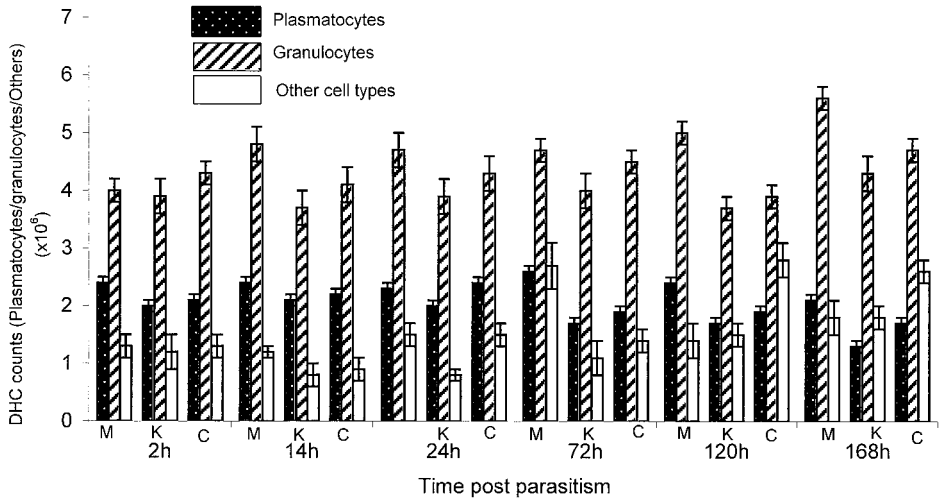


Fig. 3. Proportion of granulocytes, plasmatocytes, and other cell types in *B. fusca* parasitized by M, *C. sesamiae* from Mombasa; K, *C. sesamiae* from Kitale; and C, control (unparasitized *B. fusca*).

day 12, when the parasitoids had emerged, relative to the unparasitized larvae. There was no difference between the average weights of larvae parasitized by *C. sesamiae* from Mombasa, *C. flavipes*, and unparasitized larvae at day 12 (Fig. 5).

Mortality was not affected by parasitization (Table 2). On day 12, 36 of the control larvae had pupated as compared with 22 and 14 of larvae parasitized by *C. sesamiae* from Mombasa and *C. flavipes*, respectively. Parasitoids that emerged from host larvae parasitized by *C. sesamiae* from Kitale formed cocoons (Table 2). A significant proportion of the host larvae were still at the larval stage for those parasitized by *C. sesamiae* from Mombasa and *C. flavipes* at day 12 (Table 2).

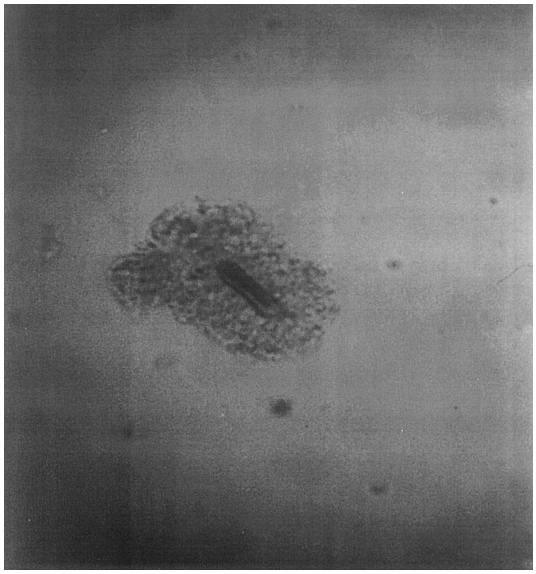


Fig. 4. Egg of *C. sesamiae* from Mombasa encapsulated in *B. fusca.*

Discussion

In this study, we examined and compared hemocytic changes in *B. fusca* larvae caused by parasitism of two biotypes of *C. sesamiae.* We investigated the possible roles played by plasmatocytes and granulocytes in the suppression of encapsulation associated with parasitism by *C. sesamiae* from Kitale and encapsulation of eggs in the case of parasitism by *C. sesamiae* from Mombasa. Findings here may contribute in providing explanation for the observed differences in abilities of the two populations to evade the *B. fusca*'s immune system. On the implications as biocontrol agent, this study suggests that if *C. sesamiae* from Mombasa is released in areas where *B. fusca* is the predominant stem borer species, this parasitoid will fail to establish. In addition, this should provide a unique way to identify more efficient natural populations of *C. sesamiae.*

The immune system of *B. fusca* was suppressed by the *C. sesamiae* population from Kitale and responded to parasitism with a decrease in THC compared with the control. This agrees with the findings of Prevost et al. (1990), who reported a reduction in the THC of susceptible *Spodoptera frugiperda* (Smith) larvae parasitized by *Campoletis sonorensis* (Cameron). The increase in the THC of *B. fusca* parasitized by the *C. sesamiae* population from Mombasa may have been due to the release of more hemocytes into circulation. Another possible explanation is that plasmatocytes and "other cell types" that were involved in the immune reaction during the parasitoid attack were destroyed in *B. fusca* parasitized by *C. sesamiae* from Kitale, resulting in a decrease in the total hemocyte count. According to Strand and Pech (1995), Lavine and Beckage (1995) hemocyte counts decreased following successful parasitization. They reported that hemocytes were induced to undergo apoptosis, leading to a reduction in the number of cells in circulation,

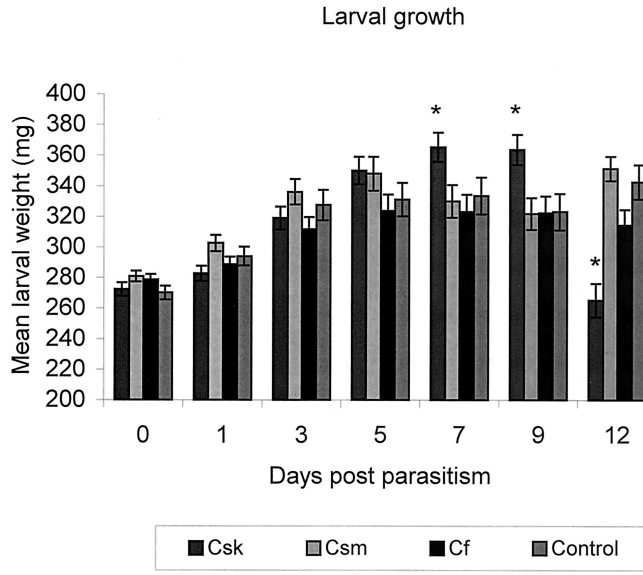


Fig. 5. Mean weights (mg ± SE) of fourth-instar B. fusca parasitized by two biotypes of *C. sesamiae* and *C. flavipes*. Control was unparasitized larvae. * Significantly different from control at $P < 0.05$.

which inhibits encapsulation. Several authors have reported on the effects of parasitism on THC in different insect hosts. In the majority of cases, the THC was lower in successfully parasitized hosts than in hosts which encapsulated parasitoid eggs (Table 3). The significant interaction between time and population further indicates that the number of hemocytes per ml over the 7-d period differed between the two populations. Strand and Pech (1995) and Ngi-Song (1995) reported that the number of hemocytes in circulation varied during growth and development. In some insect groups, there is a progressive increase in THC during larval life, with a decline after each molt, followed by an increase as growth is renewed. THC may also vary with the treatment of insects and physiological conditions (Shapiro 1979, Yeargan and Braman 1989). During the developmental period of the host, *Drosophila algoquin* Sturtevant and Dobzhansky (Diptera: Drosophididae), some cells gradually transformed through stages to form different hemocyte types (Nappi 1973).

Plasmatocytes have been reported to be involved in capsule formation in many insects (Ratcliffe and Rowley 1979, Gotz 1986, Ratcliffe 1986). It appeared that the suppression of encapsulation in this study was the decrease in the plasmatocytes, which were slightly reduced in larvae stung by *C. sesamiae* from Kitale from 72 h onward; this in addition to the reduction in numbers of the "other cell types" was also noted (Fig. 3). The number of granulocytes did not differ much. Strand and Pech (1995) observed that the granular cells were selectively targeted to undergo apoptosis. Thus, our observation of a decrease in plasmatocytes in larvae parasitized by *C. sesamiae* from Kitale may support the hypothesis that plasmatocytes are targeted for destruction in cases of successful parasitism because of the expression of polydnavirus gene products, which induce hemocyte apoptosis. A similar observation was reported by Nappi and Stream (1969), when the parasitoid, *Leptohilina heterotoma* (Thomson) (Hymenoptera: Eucoilidae) attacked and successfully parasitized *Drosophila* spp. larvae. Parasitism led to an increase in the crystal cells and a decrease in lamellocytes, suggesting that the parasitoid had suppressed the immune response. In the same study, the effect was more dramatic in resistant hosts (hosts that

Table 2. Fate of fourth-instar larvae of *B. fusca* parasitized by two biotypes of *C. sesamiae*, *C. flavipes*, and unparasitized *B. fusca* larvae

Host/parasitoid	Fate of host larvae on day 12				
	No. parasitized	No. pupated	No. still at larval stage	No. dead	No. successfully parasitized
B. fusca-CSK parasitized	51	2 (3.9)	7 (13.7)	6 (11.8)	36 (70.6)
B. fusca-CSM parasitized	51	22 (43.1)	25 (49.0)	4 (7.8)	—
B. fusca-CF parasitized	51	14 (27.5)	27 (52.9)	10 (19.6)	—
B. fusca-unparasitized (Control)	51	36 (70.6)	13 (25.5)	2 (3.9)	—

Numbers in parenthesis are percentages. Chi-square test for equal proportions: Number pupated, χ^2 value = 34.5, df = 3, P = 0.001. Number still at larval stage, χ^2 value = 14.6, df = 3, P = 0.002. Number dead, χ^2 value = 6.4, df = 3, P = 0.10 CSK, *C. sesamiae* from Mombasa; CSM, *C. sesamiae* from Mombasa; CF, *C. flavipes*

Table 3. Effects of parasitism on the THC that have been reported during parasitism from different host–parasitoid systems

Host	Parasitoid	Effects of parasitism on THC	Outcome of parasitization	Reference
Drosophila melanogaster	*Pseudeucoila bochei*	Increase	Successful parasitization	Walker 1959
Drosophila melanogaster	*Pseudeucoila mellipes*	Increase	Encapsulation	Nappi and Stream 1969
Drosophila melanogaster	*Asobara tabida*	Increase	Successful parasitization	Nappi 1981
Drosophila melanogaster	*Leptopilina heterotama*	Decrease	Successful parasitization	Rizki and Rizki 1984
Heliothis virescens	*Campoletis sonorensis*	Decrease	Successful parasitization	Davies et al. 1987
Orgyia leucostigma	*Cotesia melanoscela*	Decrease	Successful parasitization	Guzo and Stoltz 1987
Spodoptera frugiperda	*Campoletis sonorensis*	Decrease	Successful parasitization	Prevost et al. 1990
Pseudoplusia includens	*Microplitis demolitor*	Increase	Successful parasitization	Strand and Noda 1991
Pseudaltia separatela	*Cotesia kariyai*	Decrease	Successful parasitization	Yamanaka et al. 1996
Spodoptera littoralis	*Chelonus inanitus*	Decrease	Successful parasitization	Stettler 1998

mounted an effective defense reaction) in which a marked increase in the total hemocyte population was observed, followed by a decrease in crystal cells and then a precocious increase in lamellocytes (Walker 1959, Nappi and Stream 1969). A different finding, however, was noted for *Locusta migratoria* (L.) (Orthoptera: Acrididae) and *Melolontha melolontha* (L.) (Coleoptera: Scarabaeidae). Encapsulation in these host species was mediated by granular cells alone (Brehelin et al. 1989), whereas studies by Pech and Strand (1996) and Strand and Clark (1999) indicated that in *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae), neither granulocytes nor plasmatocytes are capable of forming capsules independently. However, plasmatocytes encapsulate targets if they are preincubated in medium conditioned by granular cells.

Currently, we can only speculate on the mechanism responsible for the different host responses observed to the two parasitoid populations. In many braconid and ichneumonid parasitoids, some of the most potent weapons used in disarming their host defenses are polydnaviruses (PDV) (Vinson 1990; Schmidt and Theopold 1991; Beckage 1997, 1998), which are injected by the female parasitoid into the host hemocoel (Lavine and Beckage 1995). They have been shown to cause most of the physiological effects in the host, such as changes in growth, development, behavior, and hemocytic activity (Davies et al. 1987, Vinson et al. 1979, Vinson and Stoltz 1986). PDVs have been found in *C. sesamiae* (AJN-S, unpublished). Viruses released from the calyx fluid of *C. sesamiae* from Kitale might have selectively destroyed or altered the hemocytes involved in encapsulation. According to Strand and Pech (1995), similar alterations occurred in hosts parasitized by PDV-carrying wasps, and the most direct way of preventing encapsulation is to destroy, deplete from circulation, or alter the behavior of the hemocytes that mediate encapsulation. The fact that the total hemocyte count increased in interactions in which parasitoids was encapsulated may be caused by either mobilization of differential plasmatocytes and granulocytes from hemopoietic tissues, or a stationary effect on differentiation of these cells from prohaemocytes. The current study also lends support to the view of Ratcliffe and Rowley (1979) that the cells of the plasmatocyte–granulocyte type play a major role in immunoresponse of insects, mediating whether the parasitoid eggs/larvae survive and develop, or are encapsulated.

In a previous study, *C. sesamiae* from Kitale was successfully reared on *B. fusca*, but not *C. sesamiae* from Mombasa or *C. flavipes* (Ngi-Song 1995). One possible explanation for this result is that PDV of *C. sesamiae* from Kitale is effective in suppressing the immune system of *B. fusca*, whereas the PDVs from *C. sesamiae* from Mombasa and *C. flavipes* are not because all the eggs deposited were encapsulated. Thus, it was hypothesized that the PDV associated with *C. sesamiae* from Kitale has an immunosuppressive effect on *B. fusca* leading to the development of parasitoids. but PDVs from *C. sesamiae* from Mombasa and *C. flavipes* are ineffective in this host. Our results clearly showed that parasitization by both *C. sesamiae* populations and C. *flavipes* had different effects on growth and development in the natural host (for *C. sesamiae* from Kitale) and atypical host (for *C. flavipes*), *B. fusca.* Thus, our results agree with earlier reports on the effects of parasitoids on host larvae (Gupta and Ferkovich 1998).

In general, braconids with PDV all reduced the growth and development of their typical host and prolonged the larval stage. In the interaction of *C. sesamiae* from Mombasa and *C. flavipes* with *B. fusca*, it is difficult to explain how parasitization, even though no parasitoids developed, altered the growth and physiology of the hosts. A similar observation has been reported in *Microplitis croceipes* (Cresson) with the atypical host *Galleria mellonella* (L.) (Gupta and Ferkovich 1998). Several authors have contended that the virus may interfere with endocrine or metabolic functions (Beckage 1985, Davies et al. 1987, Lawrence 1991, Strand and Dover 1991, Strand and Noda 1991, Fleming 1992). Developmental arrest in lepidopteran hosts is mediated through the endocrine system. According to Beckage (1997), the concentration of a key hormone regulating metamorphosis was disturbed after parasitism of tobacco hornworm, *Manduca sexta* (L.) by *Cotesia congregata* (Say). In that study, the level of juvenile hormone (JH) was dramatically elevated in parasitized hosts, never descending to the low level needed for pupation. The high levels of JH are probably caused by lack of sufficient juvenile hormone esterase, an enzyme that clears JH from the organism. Parasitism apparently leads to sustained low

esterase levels that prevent pupation even after the departure of the wasps from host.

Species of entomophagous insects, like other animals, exist in nature as groups of populations, with each population differing to a greater or lesser extent from others. Many of these population differences are the result of local variations in the environment, which influences allocation of gene alleles in the individuals making up these populations. *B. fusca* does not occur in the coastal area of Kenya, and thus, the *C. sesamiae* population at the coast is under no selection pressure to develop mechanisms to avoid encapsulation in this host. These population differences, because they are genetically inherited, tend to persist in time. As suggested by Diehl and Bush (1984), it is essential to know the different traits that characterize biotypes and the genetic basis of these traits if we want to preserve or augment their efficacy as biological control agents. In future work, the genetic differences between the PDVs of these two populations of *C. sesamiae* and that of *C. flavipes*, which exhibit differential abilities to suppress the immune system of *B. fusca*, will be examined.

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