Possible bases of pseudoparasitism in *Spodoptera littoralis* larvae stung by *Microplitis rufiventris*

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Received 5 June 1999; received in revised form 25 November 1999; accepted 17 January 2000

Abstract

The effects of host age and parasitoid female age on the occurrence of ‘Pseudoparasitism’, using the *Spodoptera littoralis*–*Microplitis rufiventris* host–parasitoid system were investigated. The first four larval instars of the host are not equally suitable for parasitoid development. The proportion of pseudoparasitized hosts significantly increases when: (1) the age of the female parasitoid increases; (2) oviposition occurs mostly in fourth instar larvae; (3) a later age of the host instar is used; (4) the mandibles of the newly hatched parasitoid larvae mistakenly attack host interior organs (e.g. Malpighian tubules); and (5) an imperfect growth pattern of teratocytes occurs. The reluctance of female wasps to parasitize fourth instar host larvae is not due to the thickness of host cuticle but possibly due to the unfavourable physiological state of the host larvae. The age of host larvae at the time of parasitization may influence the adverse effects of parasitoid factors (e.g. polydnavirus, venom and teratocytes) on the growth of host larvae. It is suggested that females of *M. rufiventris* are able to determine the suitability of a potential host instar for the development of their offspring. The cell diameter of *M. rufiventris* teratocytes increases with increasing age of host larvae at the time of oviposition. The association within the host of living parasitoid larvae and functional teratocytes may be important for the survival of each other and consequently for successful parasitism. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Microplitis rufiventris*; *Spodoptera littoralis*; Instar; Age; Teratocyte

1. Introduction

The physiological interactions between parasitoids and their hosts are not only complex but each association appears to be unique (Vinson and Iwantsch, 1980; Stoltz, 1986). For example, in some parasitoid–host systems the parasitized host does not grow, in others the host continues to grow and develop but at a reduced rate, and in yet others, the host grows larger than unparasitized cohorts (Stoltz, 1986). Lawrence (1986) classified insect endoparasitoids into two classes, based on the interaction between host and parasitoid. Regulators are parasitoids that alter their host’s development in order to optimize the parasitoid’s development. Conformers coordinate their larval moults and/or emergence with their host’s larval or pupal moult (Beckage and Templeton, 1986; Lawrence, 1986). *Microplitis rufiventris* Kok. is a solitary larval endoparasitoid that belongs to the first class. It attacks young instars of several economically important lepidopterans, including the cotton leafworm *Spodoptera littoralis* (Boisd.); the lesser cotton worm *S. exigua* Hbn. and the American bollworm *Heliothis armigera* Hbn. (Kokujev, 1914; El-Minshawy, 1963). Larvae parasitized by *M. rufiventris* exhibit reduced growth and are developmentally arrested (Hegazi, 1986). Similar results were reported for *Heliothis zea* (Boddie) parasitized by another braconid species of the same genus *M. croceipes* (Jones and Lewis, 1971). The source of the factors responsible for these host changes differs among the parasitoid taxa (Vinson, 1988; Stoltz, 1986; Vinson and Barbosa, 1987). These changes have been considered to result from either direct feeding and secretions by parasitoid larvae within the host and/or some factors injected by the adult female during oviposition (Edson et al., 1981; Rizki and Rizki, 1984; Dover et al. 1987, 1988).

It was often observed that some of *S. littoralis* larvae stung by *M. rufiventris* developed to an intermediate size
and then ceased development as larvae, but yet contained
no live or functional parasitoid. This has been termed
‘pseudoparasitism’ by Jones (1985) and Jones et al.
(1986). To better understand the relationships between
M. rufiventris and S. littoralis larvae, we studied the
effect of the female parasitoid’s age on the suitability of
different host instars and ages within each instar for the
subsequent development of her progeny and effects on
the host larvae. The information obtained would contrib-
ute to developing knowledge of the mechanism(s) that
lead to the phenomenon of pseudoparasitism.

2. Materials and methods

Cultures of the parasitoid Microplitis rufiventris Kok.
were kept on larvae of Spodoptera littoralis (Boisd.) at
27±1°C, 65±5% R.H. and a photoperiod of LD: 14:10
h. Both populations were reared following the methods
developed in the Department of Economic Entomology
in Alexandria (Hegazi et al., 1977; Hegazi and El-Min-
shawy, 1979). Infusions of field-collected insects were
made for both cultures.

Mating in M. rufiventris wasps occurs as soon as both
sexes are put in the presence of one another (Hegazi,
1977), thus couples of newly emerged females held
together in glass vials (25×100 mm) for 48 h were pre-
sumed mated. Also, they were provided daily with fine
droplets of honey to ensure maximum reproductive suc-
cess.

The following experimental procedures were used to
determine whether the developmental state (and possibly
the physiological state) of S. littoralis larvae has some
influence on the development of the endoparasitoid M.
rufiventris. The host larvae were grouped into instars and
ages within the instar; i.e. early second, third and fourth
instar (determined by the presence of a moulting head
capsule) and late first, second, third and fourth instar
(determined by their colour and weight). For each group
of host larvae, 6–8 2-d-old mated female parasitoids
which had no previous contact with host larvae were
used singly and each served as a replicate. Oviposition
by the female was performed every other day from day
2 post-emergence onwards and was induced by placing
the female wasp in a glass vial (7.0×2.7 cm) and tapping
the female into contact with the host larva which was
removed immediately after a single oviposition (used to
enhance precision in the procedure). Larvae that were
accidentally parasitized more than once were discarded.
Oviposition period/female was ca 3 h. The stung larvae
were observed daily and the emergence of the parasitoid
was carefully noted. ‘Hosts’ showing no emergence after
11 days were dissected in insect Ringer’s solution to ver-
ify that parasitization had occurred and that teratocytes
(derived from the parasitoid’s embryonic membrane)
were present. We also determined if developing or
encapsulated parasitoids were present in the haemocoel.
The precise number of stung hosts per female was
determined throughout the female’s life. ‘Hosts’ which
exhibited no reduced growth, lacked parasitoids and tera-
tocytes, were presumed to have escaped parasitization
and were discarded. Hosts that exhibited arrested develop-
ment and reduced growth, but contained no parasitoid
or teratocytes, were considered as ‘pseudoparasitized’,
i.e. possibly injected with polydnaviruses/venom only.

To determine if the size of host larvae at the time of
parasitization has an influence on the growth of M.
rufiventris teratocytes, the above experiments were
repeated using the above-mentioned seven age classes
of host larvae (20–25 larvae/case). At the completion of
parasitoid development (confirmed by dissection of the
host larva after bleeding) the host larvae were first
warmed to 60°C for 1 min to inhibit melanization. Pre-
liminary tests proved that this procedure had no effects
on the teratocytes at that temperature. Then the larvae
were bled through one of the first two abdominal legs.
The first one or two drops of haemolymph were collected
on a depression slide. In all cases, 50 teratocytes per host
larva were selected at random and their diameters were
measured using a compound microscope equipped with
an optical micrometer. For elliptical cells both length
and width diameters were measured and the average was
used to estimate the cell diameter. The same procedure
was carried out for hosts (day 9 post-parasitism) having
no live or functional parasitoid (i.e. pseudoparasitized).
After haemolymph removal from these pseudoparasi-
itized hosts, they were re-investigated and dissected under
a binocular microscope in order to record the presence
and condition of the parasitoid larvae.

Data were subjected to analysis of variance for deter-
mination of differences between means. Where signifi-
cant differences occurred, Duncan’s multiple range test
or Student’s t-test were applied for mean separation. The
percentages were transformed to log10 and arcsin,
respectively before statistical analysis was performed.

3. Results and discussion

In the laboratory, M. rufiventris females were able to
sting seven age classes of the first four instars of S.
littoralis larvae namely late first, early second, late second,
early third, late third, early fourth and late fourth when
larvae of each class were individually presented to them.
The mean number (±S.E.) of stung larvae/female/3 h
during the first five oviposition times of the female’s life
was 22.9±3.2, 24.3±0.8, 27.4±1.5, 27.1±3.8, 20.2±2.1,
18.7±2.1 and 17.2±2.6, respectively; i.e. the wasps stung
more late second and early third instar host larvae than
late first or early second instar hosts. However, the num-
ber of host larvae stung of the first four classes of larvae
was significantly higher (P<0.05) than those stung dur-
ing their late third to late fourth instars. These differences probably reflect relatively higher acceptance of some ages of host larvae by parasitoid females than others.

The time of parasitoid development from egg to pupation in S. littoralis larvae fed artificial diet was 7–9 days when held at 27±1°C and 60±10% relative humidity. In all cases, the parasitized hosts were classified on day 7–9 post-parasitism into hosts producing parasitoids (i.e. perfect parasitized hosts ‘or type A hosts’) and hosts producing no parasitoids (i.e. imperfect parasitized hosts). The perfect parasitized hosts exhibited arrested development and reduced growth. The maximum weight of these hosts at the completion of parasitoid development is shown in Fig. 1. The data suggests that the age of host larva at the time of parasitism influences the severity of the parasitoid’s effect on the growth of host larvae, i.e. on the degree of developmental arrest.

The imperfect parasitized host larvae were partially developmentally arrested. This type of non-successful parasitized host larvae were observed in different proportions in all classes of test larvae. They exhibited no signs of initiating metamorphosis and contained no live or functional parasitoid and have been termed ‘pseudoparasitized’ larvae (Jones, 1985; Jones et al., 1986). The pseudoparasitized host larvae were further classified according to their weights into types B and C hosts. Fig. 2 shows the mean weight of perfect and ‘pseudoparasitized’ hosts resulted from parasitizing third and fourth instar host larvae. At the completion of parasitoid development, type A hosts showed a marked reduction ($P<0.001$) in total weight gain when compared with non-parasitized ones. Type B hosts were also developmentally arrested, but their weights were heavier than type A hosts. They contained ‘melanized’ parasitoid larva (Fig. 3[4–7]). In most cases, the larvae did not complete their second or last larval ecdysis (Hegazi and Führer, 1985) and, in some cases host materials adhered to the parasitoid’s surface (Fig. 3[7]). It seems that some parasitoid larvae lacked a trigger from the host to initiate parasitoid egression.

The development of type C hosts was partially arrested and they appeared to be non-parasitized. These host larvae attained a mean weight of 122.7±1.2 mg
(±S.E.), for those resulting from stung third instar larvae, and 155.0±3.1 mg from fourth instar larvae. The corresponding unparasitized control hosts attained maximum weights of 288.3±2.3 and 299.6±4.3 mg, respectively. Some of the type C hosts exhibited no signs of initiating metamorphosis. Dissection of this group of larvae “type C hosts” revealed the existence of encapsulated (Fig. 3[11–13, 17–19]) or free first instar parasitoid larvae. Some of these stayed alive for more than two weeks (Fig. 3[10 and 21]) in their hosts without gaining weight or moulting, and some were morphologically abnormal (Fig. 3[14–16, 20]). In rare cases some of type C hosts (0.8%; n=394) pupated or formed larval–pupal intermediate which died (Fig. 4). However, a larger proportion of type C hosts resulting from stung late fourth instar S. littoralis larvae were apparently unparasitized and developed normally. In a separate experiment, a group of these late fourth instar host larvae were stung individually by 7–9 d old mated female wasps. Since parasitoid eggs hatch 24 h after oviposition, these hosts were dissected 48 h post-parasitism. Interestingly, some of these hosts contained either an unencapsulated and dead larvae (30.2%) or eggs (15.8%). Some of these eggs had shriveled. We speculate that in these cases: (a) the nutritional requirements for embryonic development of the parasitoid were not satisfied; (b) some of the nutritional or hormonal factors were toxic to the parasitoid embryos; and (c) the osmotic pressure of host’s haemolymph was suboptimal.

Like other microgastrine braconids, M. rufiventris females introduce a symbiotic polydnavirus and venom into their hosts at oviposition. Teratocytes which arise from hatching embryos also introduce factor(s) of parasite origin into the host hemocoel. All of these types of factors have been implicated in mediating developmentally alterations that occur in parasitized hosts (Jones and Lewis, 1971; Tanaka, 1987; Stoltz et al., 1988; Tanaka and Vinson, 1991; Dover et al., 1995). The three types of different developmentally arrested host larvae (types A to C) caused by natural parasitism suggest that another factor (i.e. the parasitoid larvae) influences the degree of developmental disruption in the parasitized hosts.

The rate of production of imperfect parasitized hosts “hosts types B and C” that resulted from different classes of host larvae (late first to late fourth) parasitized at different times during the life span of the female wasp is shown in Figs. 5 and 6. The following could be suggested: (1) All classes of larvae tested were not equally suitable for M. rufiventris development. The proportion of pseudoparasitized hosts significantly increased (P<0.01) when oviposition occurred in fourth instar larvae. Weseloh (1976) also found that the gypsy moth fourth instar were less suitable for development of the braconid Cotesia melanoscelus (Ratzeburg) than earlier instars. (2) Within the same instar or age of either third or fourth instar host larvae the number of pseudoparasitized larvae increased with each subsequent increase in the age of the female wasp, i.e. the ability of M. rufiventris to develop successfully was female’s age-dependent. The phenomenon was more pronounced in later instars than earlier ones. The effect may be due to one or all of the factors (polydnavirus, venom and teratocytes) injected into the host larva at oviposition, or may be related to the dose-dependent effect of calyx fluid (Strand and Dover, 1991), the amount of which is injected may vary during the female’s life and/or when a larger host larva receives the same dose of parasitoid factors. Nevertheless, Harrison et al. (1993) reported that parasitism of Helicoverpa zea by M. croceips was successful regardless of the age of the parasitoid or of the third and fourth larval instars used. (3) Successful parasitism also may vary among ages of the same instar at oviposition. Little or no effect of parasitoid female age was observed when hosts are late first or second instars. Judging from the number of pseudoparasitized hosts, the late stages of the third or fourth instars were clearly suboptimal for parasitoid development (Figs. 5 and 6). The data indicate that the physiological state of the host at this time of parasitization is an important factor in the development of the endoparasitoid.

The age structure of the non-functional parasitoid larvae found in pseudoparasitoid hosts dissected on day 9 from oviposition in the seven classes of host larvae is shown in Fig. 7. The data show that the disturbance in parasitoid development using late third to late fourth host larvae at parasitism, is teleologically speaking to the parasitoid’s disadvantage, i.e. the host itself had an effect on the parasitoid. Stung late fourth instar S. littoralis larvae produced 41.7% non-functional parasitoid larvae, more than 50% of the latter remained as first instar in their host, i.e. the developmental state (and possibly the physiological state) of the host at the time of parasitism.

[Fig. 4. Three cases of imperfect parasitized host larvae “type C hosts”. In most cases, the larva exhibited no signs of initiating metamorphosis (1). In rare cases (0.8%), it developed into a juvenilized pupa (larval–pupal intermediate) (2) or dwarved pupa (3) compared with pupa resulting from non-parasitized host larva (4).]
may influence the severity of the host larva’s internal defences towards parasitoid larvae. Gunasena et al. (1989) reported that host fourth instar of *Heliothis virescens* gave rise to a lower percentage (16%) of emergence of *Campoletis sonorensis* than did the three younger instars (54–66%). They attributed this failure to the inability of parasitoid larvae to chew through the thickened cuticle of late fourth-instar hosts or to the lack of a trigger from the host to initiate parasitoid egression. In the *S. littoralis–M. rufiventris* system, the elimination of 21.1% of parasitoids as first instar larvae when the late fourth instar host larvae were stung by female wasps, suggests in this case that there is no relation with thickened cuticle or any trigger to initiate parasitoid emergence. Also, the inability of more than 40% of parasitoid larvae to develop successfully in the late fourth instar larvae suggests why *M. rufiventris* may avoid these larvae. Parasitoid reluctance to parasitize late fourth instar (17.2±2.6 larvae/female/3 h/oviposition time) suggests that *M. rufiventris* females were able to
determine the suitability of a potential host instar for the development of her offspring. Arthur (1981) and Vinson (1990) reported that the parasitoids use a variety of cues to determine host suitability including host size, shape, texture, movement and presence or absence of internal or external chemicals.

Ables and Vinson (1981) observed that 5–10% of H. virescens larvae were pseudoparasitized by Chelonus insularis. Injection of poison gland and calyx fluid into eggs or larvae of H. virescens did not result in the expected pseudoparasitized larvae suggesting that additional factors are required. However, Dover et al. (1995) reported that M. demolitor teratocytes appear to be important in enhancing the degree of developmental arrest in H. virescens. When a M. rufiventris embryo develops at 27°C, it hatches after 24–28 h. The cells (teratocytes) which make up the extraembryonic membrane dissociate and attain their maximum size as ‘mature cells’ at the completion of parasitoid development. The growth pattern of mature M. rufiventris teratocytes in perfect parasitized hosts ‘type A hosts’ is shown in Fig. 8. The data suggest that the age of host larvae at the time of parasitism influences the subsequent growth of the teratocytes. Their cell diameter increased with increase in the age of host larvae in which oviposition had occurred.

Fig. 9 shows the growth pattern of 9 d old M. rufiventris teratocytes in pseudoparasitized hosts ‘host types B and C’ which resulted from hosts stung at the onset, or the end of fourth larval instar. When type B hosts were dissected, different classes of teratocytes sizes were found (1) small spherical cells (94–123 μm), (2) medium spherical cells (137–170 μm) and large cells (174–274 μm) of abnormal shape. Some of the latter present irregular contours, pseudopodial-like projections and ramified nuclei. These abnormalities were associated with developmental abnormalities of parasitoid larvae (Fig. 3[5–6]). The overall mean diameter of teratocytes in these type B hosts was significantly larger than the corresponding diameter of cells in type C hosts (Fig. 9). It is suggested that the larger cells might have a protective function, reducing the effects of host materials or hormones which might have toxic effects leading to developmental abnormalities in some parasitoid larvae.

In hosts of type C, the effect of host age on growth rate of both parasitoid and its teratocytes appeared to be more severe. Some hosts contained developmentally arrested first instar of parasitoid larvae (Fig. 3[9–20]). Some of the latter were partially or totally encapsulated (Fig.
and some showed developmental disruption (Fig. 3[14–16]). The co-existing teratocytes were significantly smaller in both number ($P<0.001$; data not shown) and diameter ($P<0.05$). Possible causes of the decline in teratocyte number could be the osmotic pressure of host’s haemolymph, or deterioration of the host environment which might have led to the degeneration of some cells. In this case, the host defences appear to be partially effective and both host and parasitoid later die. The fat body of these hosts was more developed than in either perfect parasitized hosts or non-stung ones. The present observations suggest that factors associated with the developing parasitoid larva ‘e.g. teratocytes’ are involved, at least in part, in the developmental alteration in host larvae. And, the existence of both living parasitoid larvae and highly functional teratocytes is important for the survival of each other. Fig. 9 shows also that the overall mean diameter of 9 d teratocytes was significantly ($P<0.01$) smaller than the corresponding cells in either hosts type A or B (Figs. 8 and 9). Vinson (1970) found that when *Cardiochiles nigriceps* teratocytes were injected into larvae of *H. virescens* they grew in the absence of larval parasitoids, reduced host growth and prevented normal host pupation. Therefore, it is speculated that the inorganic or organic constituents or both of *S. littoralis* larval haemolymph, especially those parasitized during their late fourth instar were not in favour (e.g. osmotic pressure) for teratocyte growth.

Three other observations were recorded in ‘type C hosts’, especially those stung when second or third instar larvae. In a rare single case, a host larva day-9 post-parasitism was teratocyte-free and the parasitoid larva was still alive as a very young first instar. This observation may reflect the importance of teratocytes for parasitoid development. In the second case, a young parasitoid larva was found dead in the host’s head capsule for an unknown reason. In the third, and most important, observation accidental elimination of the young parasitoid larvae was found to be a factor which may lead to pseudoparasitism. In this case, the host’s Malpighian tubules were injured, possibly in error as a result of attack by the sickle-shaped mandibles of the newly hatched parasitoid larva. In this case, capsules of host blood cells were formed immediately over the wounds (Fig. 3[8]) and in some cases the parasitoid larva was either included in these capsules or suspended free (Fig. 3[9,10]). In these cases, the cell diameter of the teratocytes was as in hosts type A or B.

**Acknowledgements**

The authors thank anonymous reviewer(s) for constructive comments on the manuscript. The first author wishes to thank the Alexander von Humboldt Foundation for the research scientific donation used in this work.

**References**


