Parasitization of *Helicoverpa zea* (Lepidoptera: Noctuidae) by *Palexorista laxa* (Diptera: Tachinidae): Influence of Host Developmental Stage on Host Suitability and Progeny Production[1]

W. R. Martin, Jr.[2], Donald A. Nordlund[3], and William C. Nettles, Jr.[3]

Pest Management Research Unit, USDA-ARS, Route 5, Box 808
College Station, TX 77840

J. Entomol. Sci. 27(2):164 -171 (April 1992)

ABSTRACT *Palexorista laxa* (Curran) (Diptera: Tachinidae) successfully parasitized 2nd through 5th instars and prepupal *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in the laboratory. Percent successful parasitization (that which resulted in the production of adult parasitoid progeny) increased with host developmental stage, reaching 97% in 5th instars, but fell to 75% in prepupae. The mean number of fly puparia and adults produced per successfully parasitized larva was greatest in 5th instar hosts, reaching 7.8 ± 0.6 (SEM) and 5.5 ± 0.4, respectively. The sex ratio of adult parasitoid progeny per host larval stadium was variable. Host favorability indices were calculated for each host stadium, and was highest in 5th instars. This index allows a direct comparison of the overall impact and reproductive potential of *P. laxa* attacking hosts of varying developmental stages.

KEY WORDS Insecta, *Helicoverpa zea, Palexorista laxa,* parasitoid-host interaction.

Many parasitoids will attack only a narrow range of host species. Further, certain developmental stages of a given host species are generally preferred over others (Lewis 1970, Hopper and King 1984). Many factors regulate this host range, including spatial and temporal synchrony of the parasitoid and its host, host defense mechanisms, and adequacy of nutritional resources (Vinson and Iwantsch 1980). Frequently, parasitoids are evaluated as biological control agents simply according to their ability to parasitize available hosts. However, the success of a parasitoid may depend on, among other things, the age structure of the host population (Doutt et al. 1976). Many studies have shown that the age structure of a host population can influence percent parasitization, parasitoid development rate, host mortality, and the production of parasitoid progeny, in Hymenoptera (Lewis 1970, Jones and Lewis 1971, Beckage and Riddiford 1978, Nechols and Kikuchi 1985) and, less commonly, in Diptera (Miles and King 1975, Mani and Nagarkatti 1981, Ramadhane et al. 1987, Martin et al. 1989a). Information on how a parasitoid interacts with its host is important in developing management programs, and in properly evaluating the true impact of the parasitoid on a population of hosts.

---

[1] Accepted for publication 11 February 1992.

[2] Present Address: BIOSYS, Inc., 1 Progress Blvd., Box 26, Alachua, FL 32615.

[3] Present Address: USDA, ARS, Subtropical Agricultural Research Laboratory, Biological Control of Pests Research Unit, 2413 East Hwy 83, Weslaco, TX 78596.

The tachinid parasitoid *Palexorista laxa* (Curran), a native of India and Africa, is reported to attack primarily mid- to late-instar *Heliothis armigera* (Hübner) larvae (Jackson et al. 1976). *P. laxa* was imported into the United States as a potential biological control agent for *Helicoverpa* spp., especially the cotton bollworm, *Helicoverpa zea* (Boddie), and has shown promising results in field cage tests (Jackson et al. 1976). All published accounts on the biology of this parasitoid have utilized only large larvae (4th - 6th instars) as hosts. Thus, little is known of the interaction between this parasitoid and smaller host larvae. This study was initiated to determine how host developmental stage can effect rates of parasitization, host mortality, and progeny production by *P. laxa*.

Materials and Methods

The colony of *P. laxa* used in this study was established from a colony maintained by J. E. Powell at the USDA Delta States Research Center, Stoneville, MS. The insect was originally brought to this country in the mid 1970's by M. Shepard, Clemson University. The experiments reported here were conducted at the USDA, ARS, Pest Management Research Unit in College Station, TX. *Palexorista laxa* were reared on larvae of the cotton bollworm, *H. zea,* using the procedure employed for another tachinid parasitoid, *Eucelatoria bryani* Sabrosky (Nettles 1980). The rearing room, in which all experiments were conducted, was maintained at 27°C, 23 - 60% RH and a photoperiod of 14:10 (L:D). *Helicoverpa zea* eggs were obtained in weekly shipments from Stoneville, MS. Larvae were reared in large grids on an agar-based Nutrisoy® diet (Shaver and Raulston 1971).

For each experiment, 10 *H. zea* larvae of the same age and developmental stage (instars 1 - 5 and prepupae) were exposed to a group of 20 randomly selected female flies of normal size, age 7-9 days, in a Plexiglas® cage (25 X 25 X 25 cm). Flies were nitrogen-anesthetized, sexed, and placed into the cage at least 2 h prior to the introduction of host larvae to allow recovery from anesthesia.

Helicoverpa zea larvae are cannibalistic. To reduce cannibalism during exposure to the parasitoid, two sections (23 X 13 cm) of galvanized steel hardware cloth (12.5 mm^2 openings) were placed on the bottom of the cage. These hardware cloth sections provided the larvae a three dimensional structure to crawl on. The increased surface area reduced contact between larvae in the cage, but allowed the parasitoids access to the larvae. Although experiments were conducted with overhead lighting turned off, substantial sunlight did enter through a window located at one end of the room. Because adults of *P. laxa* are positively phototactic, the cage was rotated on a turntable operating at 1 rpm to ensure an even distribution of the flies within the cage.

Larvae were exposed to high numbers of parasitoids, with no measures taken to prevent superparasitization, to maximize the probability of obtaining 100% parasitization. Our goal was to collect data on oviposition, progeny production, sex ratios, etc. of *P. laxa* on hosts of different life stages. Low or moderate rates of parasitization would have made this much more difficult.

After a 2 h exposure period, larvae were removed, nitrogen-anesthetized, and placed on a cold plate, where they were examined under magnification for the presence of *P. laxa* eggs. This procedure was to confirm that the parasitoids were ovipositing. Larvae were then placed in individual 30 ml plastic cups containing Nutrisoy® diet (Shaver & Raulston 1971). Space in the rearing room used for these experiments was limited, thus these cups were held in a room maintained at 26°C, ambient RH, and a photoperiod of 12:12 (L:D). These minor changes in environmental conditions were not expected to effect parasitoid development.

The condition of each larva was recorded every 2-3 days. Upon emergence and subsequent pupation of *P. laxa* maggots, the fly puparia and the remains of the parasitized larva from each cup were transferred to a clean plastic cup. This procedure reduced fungal contamination and facilitated the counting and sexing of adult flies. *Helicoverpa zea* pupae were treated in the same manner. Insects which had not eclosed within 3 weeks of the majority of flies or moths were recorded as dead (in every case these pupae were shriveled and desiccated). Because of the wide range in size of fly puparia obtained in this study, randomly chosen puparia from 5th instar and prepupal hosts which produced ≤ 5 or ≥ 15 fly puparia were removed and weighed.

Determination of *H. zea* instar was based on age, weight, and larval length (Neunzig 1969). Four replications (10 larvae of each developmental stage per replication) were exposed to different groups of flies on separate days. The exposure period for each experiment was initiated within 6-7 h after the onset of photophase because time of day may affect the activity of some tachinids (Nettles 1979). For each experiment, a set of 10 control larvae was treated in the same manner but the larvae were not exposed to flies.

Data were analyzed using the ANOVA procedure (SAS Institute 1985) and significant differences among the mean number of progeny produced were determined by Duncan's multiple range test at the 0.05 level of significance (Duncan 1955).

Results and Discussion

Parasitization. *Palexorista laxa* successfully parasitized instars 2 - 5 as well as prepupal *H. zea* (Table 1). Successful parasitism is defined here as that resulting in the production of parasitoid progeny. Percent successful parasitization generally increased with host instar, ranging from 42.5% in 2nd instar hosts to 97.4% in 5th instar hosts, then falling to 75% in prepupae. Published reports on *P. laxa* suggest that only large (4th instar and later) hosts are attacked by this parasitoid (Chauthani and Hamm 1967, Jackson et al. 1976). Chauthani & Hamm (1967) reported that *P. laxa* successfully parasitized only "half-grown or larger" *H. zea* larvae, and that earlier instars died before the parasitoid could complete its development. The current study indicates that 2nd and 3rd instars, which may be rarely attacked in nature, are also suitable hosts.

Eggs were deposited on all host stages, but no parasitoid progeny were produced when 1st instars served as hosts. Martin et al. (1989b) recorded the numbers and location of eggs deposited by *P. laxa* on various developmental stages of *H. zea,* and noted that significantly fewer eggs were deposited on 1st instars than on any other developmental stage. It is possible that *P. laxa* females may have had difficulty locating and ovipositing on small larvae. Females of the tachinid *Voria ruralis* (Fallen) were reported as having difficulty grasping 1st

Table 1. Parasitization and mortality of different developmental stages of *H. zea* larvae attacked by the tachinid parasitoid *P. laxa* in the laboratory.

	Host Developmental Stage					
	1st	2nd	3rd	4th	5th	prepupae
# of exposed larvae failing to reach adult stage/# of exposed larvae (= MOR/N)	9/39	36/40	23/37	37/37	38/39	38/40
# of exposed larvae successfully parasitized* (=SPL)	0	17	17	29	38	35
# of control larvae failing to reach adult stage/# of control larvae (= NAT/N)	0/38	5/40	6/39	4/37	5/39	10/40
% additional parasitoid induced mortality† (= APM)	23.1	41.6	13.8	19.3	0.0	5.6

* Successful parasitism is defined as that which resulted in the production of adult parasitoid progeny.

† Mortality not due to successful parasitism, nor accounted for in control groups (see text for additional information).

instar *Trichoplusia ni* (Hübner) when attempting to oviposit (Elsey and Rabb 1970). Further, it is possible that 1st instars which were attacked did not provide adequate nutritional resources to support developing parasitoids (Vinson and Iwantsch 1980). Thus, 1st instar *H. zea* are apparently unsuitable as hosts for *P. laxa.*

The decrease in percent successful parasitization in prepupae may have been due to early pupation of some of the host larvae, preventing completion of *P. laxa* development and emergence. Several of the parasitized prepupae were dissected after pupation, and were found to contain dead *P. laxa* maggots (unpublished data). Thus, after a parasitized host pupates, *P. laxa* apparently cannot complete its development, although it can still induce host mortality. This phenomenon has also been observed in the tachinids *E. bryani* (Martin et al. 1989a) and *Lixophaga diatraeae* (Townsend) (Miles & King 1975).

Host Mortality. Parasitoid-induced host mortality without parasitoid progeny production is a common occurrence, though it can be difficult to quantify (Van Driesche 1983). This mortality is important in evaluating the impact of a parasitoid on its host population. In the present study, we observed host larval mortality which could not be attributed to natural, non-parasitoid related causes (i.e., compared to mortality of control insects), nor to developing and emerging maggots. Thus, it was apparent that this parasitoid can induce host mortality in the absence of progeny production. This additional parasitoid-induced mortality (APM) was calculated with the following formula:

$$\mathrm{APM} = (\mathrm{MOR} - \mathrm{SPL}) - [(\mathrm{MOR} - \mathrm{SPL}) \times \mathrm{NAT}],$$

where : MOR = mortality (total # of exposed host larvae failing to reach the adult stage),
SPL = # of successfully parasitized larvae, and
NAT = proportion of those larvae which died of 'natural' causes, as determined by control groups.

APM was then converted to percent by dividing by N and multiplying by 100. Percent APM was generally lowest in the larger hosts (instars 3, 4, 5 and prepupae), and highest in instars 1 and 2 (Table 1). Martin et al. (1989a) also reported the highest APM levels in *H. zea* instars 1 and 2 which were attacked by the tachinid *E. bryani*. Other investigators have reported high mortality rates among small instar hosts which are attacked by parasitoids (Elsey and Rabb 1970, Rahman 1970).

In general, the mean number of *P. laxa* puparia and adults produced per successfully parasitized larva increased steadily with the developmental stage of the host, reaching a maximum in 5th instars and prepupae (Table 2). This indicates that larger hosts were attacked more frequently, or that larger hosts can support the development of more parasitoids (or both). Martin et al. (1989b), however, found that *P. laxa* deposited significantly more eggs on 4th instars than on any other developmental stage. The increase in progeny production associated with increasing developmental stage seen in the present study thus is correlated with an increase in host resources available to parasitoid progeny.

Percent eclosion of flies was high (90%) in host instars 2 and 3, then decreased in instars 4 and 5 and prepupae (Table 2). This decrease in pupal eclosion seen in later developmental stages may have been due to superparasitism in the larger hosts, which resulted in higher pupal mortality. In 5th instar and prepupal hosts, this overcrowding resulted in the production of fly puparia which weighed significantly less than puparia obtained from hosts which produced fewer progeny ($F = 1230.83$; $df = 1, 170$; $P << 0.01$). Puparia from 5th instars and prepupae producing ≤ 5 puparia weighed an average of 42.9 ± 1.0 mg (SEM), while those from 5th instars and prepupae producing ≥ 15 puparia weighed an average of 10.2 ± 0.3 mg. Martin et al. (1989a) and Mani and Nagarkatti (1981) also reported a decrease in puparial weight for *E. bryani* as the number of puparia per host increased. Despite this decrease in percent eclosion of puparia from 5th instars and prepupae, significantly more adults were produced per successfully parasitized late instars (5th and prepupae) than from earlier instars (Table 2).

Sex Ratios. Sex allocation in dipterous parasitoids is poorly understood (Waage 1986). In the current study, sex ratios of adult *P. laxa* emerging from each developmental stage were quite variable (Table 2). Under normal rearing conditions in our laboratory, the female: male ratio of *P. laxa* is slightly male biased (0.81). This value was calculated from a random sample of 5663 flies, collected from 16 cages. Martin et al. (1989a) and Mani and Nagarkatti (1981) reported a similar sex ratio for *E. bryani* under normal rearing conditions. Ziser et al. (1977) found that the proportion of male *Eucelatoria* sp. obtained from 5th instar *Helicoverpa virescens* (F.) increased significantly as the number of maggots/host increased. This suggests that larval overcrowding may favor the survival of males, which has been reported in some Hymenoptera (Waage 1986). However, this trend has not been seen in other tachinids (Adam and Watson 1971, Étienne 1969). Mani and Nagarkatti (1981) obtained highly male biased sex ratios of *E. bryani* from smaller hosts (3rd and 4th instars), and only slightly male biased sex ratios from early and late 5th instars.

Table 2. Progeny production by the tachinid parasitoid *P. laxa* attacking different developmental stages of *H. zea* larvae in the laboratory.

	Host Developmental Stage					
	1st	2nd	3rd	4th	5th	prepupae
Mean # of fly pupae per successfully parasitized larva* ± SEM (Data extremes)	0 a†	1.8 b ± 0.2 (1-4)	2.0 b ±0.4 (1-6)	2.6 b ±0.4 (1-8)	7.8 c ±0.6 (2-17)	7.3 c ±1.1 (1-25)
% eclosion (= HAT)	–	90	91	79	71	64
mean # of adult flies per succesfully parasitized larva ± SEM (= AFP) (data extremes)	0 a	1.6 b ±0.2 (1-4)	1.8 b ±0.3 (1-5)	2.2 b ±0.3 (1-5)	5.5 c ±0.4 (2-11)	4.7 c ±0.6 (1-18)
Sex ratio of adult flies (F/M) (= SRA)	–	0.65	1.29	0.73	1.12	0.98

* Successful parasitism is defined as that which resulted in the production of adult parasitoid progeny.

† Means followed by the same letter within a row are not significantly different (P = 0.05; Duncan's multiple range test).

Host Favorability Index. The overall impact of a biological control agent on its host population should not be evaluated on the basis of a single parameter, but rather on a group of elements. The present study indicates that host developmental stage can strongly influence rates of successful parasitism, parasitoid-induced mortality, progeny production, and other aspects of the reproductive and developmental biology of *P. laxa.* The 'host favorability index' (HFI) developed by Martin et al. (1989a) takes many of the above components into account and is based on the ability of the parasitoid to successfully parasitize its host, induce host mortality, and reproduce itself.

Host favorability indices were calculated for each of the 6 host instars and are presented graphically in Fig. 1. The highest indices were associated with 5th instars and prepupae, probably because of their ability to contribute most to parasitoid population growth. Martin et al. (1989a) also calculated the highest HFI values for 5th instar and prepupal hosts attacked by *E. bryani.*

Jackson et al. (1976) reported that *P. laxa* is capable of 51% parasitism of *H. virescens* in field cage experiments. Data from the present study indicate that the optimum time for field release of *P. laxa* is when large (4th instar and larger) *H. zea* larvae are present for parasitization. These large larvae are more readily attacked (at least, when the parasitoid can access these larvae), and can sustain the development of more parasitoids than smaller larvae. Large *H. zea* larvae are generally more difficult to control (chemically and biologically), in part because they are often found inside plant fruiting bodies. However, they do leave these protected sites to burrow into the ground to pupate. During this time, the larvae are accessible to the parasitoid. Thus, *P. laxa* may be an important component of a *Helicoverpa* population suppression program.

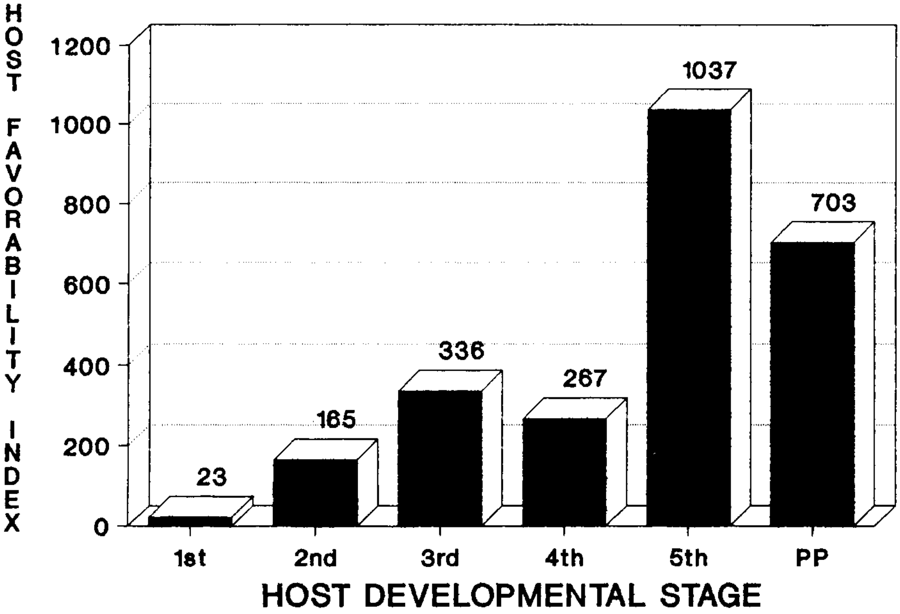

Fig. 1. Host favorability indices calculated for different developmental stages of larval *Helicoverpa zea* when exposed to the parasitoid *Palexorista laxa*.

Acknowledgment

We thank J. Bernardino and C. Menn for maintaining colonies of the parasitoid. B.A. Dover, C.G. Jackson, and G.S. Nuessly provided helpful reviews of the manuscript. Mention of a proprietary product does not constitute an endorsement of the product by the USDA.

References Cited

Adam, D. S. and T. F. Watson. 1971. Adult biology of *Exorista mella.* Ann. Entomol. Soc. Am. 64: 146-149.

Beckage, N. E. and L. M. Riddiford. 1978. Developmental interactions between the tobacco hornworm, *Manduca sexta,* and its braconid parasite, *Apanteles congregatus.* Entomol. Exp. Appl. 23: 139-151.

Chauthani, A. R. and J. J. Hamm. 1967. Biology of the exotic parasite *Drino munda* (Diptera: Tachinidae). Ann. Entomol. Soc. Am. 60: 373-376.

Doutt, R. L., D. P. Annecke and E. Tremblay. 1976. Biology and host relationships of parasitoids. *In*: C. B. Huffaker and P. S. Messenger (eds), Theory and Practice of Biological Control. Academic Press, New York: 788 pp.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

Elsey, K. D. and R. L. Rabb. 1970. Biology of *Voria ruralis* (Diptera: Tachinidae). Ann. Entomol. Soc. Am. 63: 216-222.

Étienne, J. 1969. Étude en laboratoire de la tachinaire *Diatraephaga strialis* Townsend, parasite naturel de *Chilo sacchariphagus* Bojer. Entomophaga 14: 407-419.

Hopper, K. R. and E. G. King. 1984. Preference of *Microplitis croceipes* (Hymenoptera: Braconidae) for instars and species of *Heliothis* (Lepidoptera: Noctuidae). Environ. Entomol. 13: 1145-1150.

Jackson, C. G., D. E. Bryan, E. G. Neeman and R. Patana. 1976. *Palexorista laxa:* Development, longevity, and production of progeny on *Heliothis* spp. Environ. Entomol. 5: 431-434.

Jones, R. L. and W. J. Lewis. 1971. Physiology of the host-parasite relationship between *Heliothis zea* and *Microplitis croceipes*. J. Ins. Physiol. 17: 921-927.

Lewis, W. J. 1970. Study of species and instars of larval *Heliothis* parasitized by *Microplitis croceipes*. J. Econ. Entomol. 63: 363- 365.

Mani, M. and S. Nagarkatti. 1981. Development of *Eucelatoria* sp. near *armigera* (Coq.) on instars of *Heliothis armigera* (Hübn.). Entomol. 6: 335-341.

Martin, W. R., Jr., D. A. Nordlund and W. C. Nettles, Jr. 1989a. Influence of host developmental state on host suitability and reproductive biology of the parasitoid *Eucelatoria bryani*. Entomol. Exp. Appl. 50: 141-147.

1989b. Ovipositional behavior of the parasitoid *Palexorista laxa* (Diptera: Tachinidae) on *Heliothis zea* (Lepidoptera: Noctuidae) larvae. J. Entomol. Sci. 24: 460-464.

Miles, L. R. and E. G. King. 1975. Development of the tachinid parasite, *Lixophaga diatraeae,* on various developmental stages of the sugarcane borer in the laboratory. Environ. Entomol. 4: 811-814.

Nechols, J. R. and R. S. Kikuchi. 1985. Host selection of the spherical mealybug (Homoptera: Pseudococcidae) by *Anagyrus indicus* (Hymenoptera: Encyrtidae): Influence of host stage on parasitoid oviposition, development, sex ratio, and survival. Environ. Entomol. 14: 32-37.

Nettles, W. C., Jr. 1979. *Eucelatoria sp.* females: factors influencing response to cotton and okra plants. Environ. Entomol. 8: 619-623.

1980. Adult *Eucelatoria* sp.: response to volatiles from cotton and okra plants and from larvae of *Heliothis virescens, Spodoptera eridania,* and *Estigmene acrea.* Environ. Entomol. 9:759-763.

Neunzig, H. H. 1969. The biology of the tobacco budworm and the corn earworm in North Carolina. North Carolina Agric. Exp. Sta. Tech. Bull. No. 169.

Rahman, M. 1970. Mutilation of the imported cabbageworm by the parasite *Apanteles rubecula.* J. Econ. Entomol. 63: 1114-1116.

Ramadhane, A., S. Grenier and G. Plantevin. 1987. Physiological interactions and development synchronizations between nondiapausing *Ostrinia nubilalis* larvae and the tachinid parasitoid *Pseudoperichaeta nigrilineata.* Entomol. Exp. Appl. 45: 157-165.

SAS Institute, Inc. 1985. SAS User's Guide: Statistics, 1985 edition. SAS Institute, Inc. Cary, N.C.

Shaver, T. N. and J. R. Raulston. 1971. A soybean-wheat germ diet for rearing the tobacco budworm. Ann. Entomol. Soc. Am. 64: 1077-1079 .

Van Driesche, R. G. 1983. Meaning of "percent parasitism" in studies of insect parasitoids. Environ. Entomol. 12: 1611-1622.

Vinson, S. B. and G. F. Iwantsch. 1980. Host suitability for insect parasitoids. Annu. Rev. Entomol. 25: 397-419.

Waage, J. 1986. Family planning in parasitoids: adaptive patterns of progeny and sex allocation. *In*: J. Waage & D. Greathead (eds), Insect Parasitoids. Academic Press, New York: 389 pp.

Ziser, S. W., J. A. Wojtowicz and W. C. Nettles, Jr. 1977. The effects of the number of maggots per host on length of development, puparial weight, and adult emergence of *Eucelatoria* sp. Ann. Entomol. Soc. Am. 70: 733-736.