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ALESSANDRO BRATTI*, W.C. NETTLES**

* Istituto di Entomologia «Guido Grandi» Università degli Studi di Bologna

** Pest Management Research Unit Southern Crops Research Laboratory, USDA, ARS, College Station, TX, USA

In vitro rearing of *Palexorista laxa* (Curran) (Diptera: Tachinidae) on haemolymph-based diets

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Introduction

Palexorista laxa (Curran), a tachinid that parasitizes *Heliothis armigera* (Hübner) in India and Africa, is being studied as a potential parasitoid of *Heliothis zea* (Boddie) and *Heliothis virescens* (F.) (Lep. Noctuidae), pests of cotton and other crops in the southern part of the United States. Data from field cage tests using 2500 insects/acre showed that *P. laxa* can attain a 51% rate of parasitization against *H. zea* and *H.virescens* (Jackson *et al.*, 1976). The importance of the potential role of *P. laxa* in biocontrol programs has caused us to attempt to rear this parasitoid *in vitro*.

Two species of tachinids have already been successfully reared on synthetic diets (Grenier *et al.*, 1978; Nettles, 1980). This paper is the first to report the results of larval rearing of *P. laxa* on diets based on haemolymph which was extracted from the larvae of *Manduca sexta* (L.).

Materials and methods

All the media tested were based on haemolymph collected from the last instar larvae of *M. sexta* reared on artificial diet. Extraction and preparation techniques are fully described in Xie *et al.*, 1986. Only haemolymph for the fourth test group was suction-extracted from the amputated thoracic legs of the larvae by a 1-cc syringe. Haemolymph in the first three tests was collected by gently squeezing larvae.

The *P. laxa* maggots were dissected from *H. zea* larvae that had been superparasitized by putting hosts in the *P. laxa* cage for 1 or 2 hours. The first instar maggots were used after having been in the host for either 4-5 or 24 hours.

There were four tests. In the first, 30 maggots were held singly in drops of haemolymph (5, 10, 20 µl) in plastic microtiter plates (MicroWell Plate 96 F

Nunclon®). The second test was comprised of 75 maggots held singly in 10 µl drops in sterile tubes (∅ 0,5 cm × h 3.5 cm) containing either haemolymph or haemolymph enriched with Nutrisoy® flour (ADM, Decatur, Illinois) at 0.26%.

In the third group, 100 maggots were divided into 4 or 5 specimens in glass petri dishes (∅ 5 cm × h 1.2 cm). Each petri dish contained a 300 mg medium of haemolymph plus varying concentrations of soy residue (SR), (Fi-Pro® TM F 200, G.P.C., Muscatine, IOWA) previously sterilized by autoclaving for 15' at 125°C. In the fourth test, 34 maggots were held individually in cells of a microtiter plate containing a diet of 80% haemolymph and 20% of the same sterile soy residue as above. Each cell contained 80 mg of soy residue and sufficient haemolymph to produce 20% SR. All biological test materials were held at 100% rh and 24-25°C and observed daily. Larval instars were determined by the form and size of the buccopharyngeal apparatus and posterior spiracles. The percentages in Table 1 were based on the number of maggots surviving the first day. The development period is based on the number of days required to reach the pupal and adult stages; average pupal weights were calculated only for the fourth test group.

Results and discussion

A very important aspect of this study is that the larval stages of *P. laxa* developed in a liquid medium composed of *M. sexta* haemolymph (Table 1, test 1). Of ten first instar maggots tested on 10 µl drops of haemolymph in wells of microtiter plates, four developed to the third instar and one to the pupal stage (test 1). Growth and development occurred because maggots were able to maintain contact with the atmosphere by elevating the tips of their long posterior everted spiracles above the surface of the liquid medium. When the test volume was 20 µl, none of the maggots developed beyond the first instar. In test 2 when the maggots were placed in small glass tubes (diameters smaller then those of microtiter plate wells) supplied with either haemolymph or haemolymph plus Nutrisoy® flour, most of the maggots developed to the second instar and died. The larvae likely died by drowning because the smaller diameter of the tubes produced higher liquid levels. When a 20% or 25% concentration of a commercially available soy residue (SR) was added to *M. sexta* haemolymph in test 3 maggots developed to the adult stage. It was the only test in which adults were obtained. SR apparently serves both as a thickener and as a source of nutrient for *P. laxa* because it has a high fiber content (37.5% insoluble and 17.5% soluble fiber) and sizable levels of proteins and minerals. Other authors used agarose or agar (Greiner *et al.* 1978, Nettles *et al.* 1980) as gelling agents for tachinid rearing *in vitro*. SR, besides having the advantage of being less expensive than agarose and agar, shows good results also for tachinids that do not posses everted spiracles: Bratti (unpublished data), has reared *Eucelatoria bryani* (Sabrowsky) first instar maggots, on medium composed of 70% *M. sexta* haemolymph and 30% SR and obtained a pupae yield of 12.5%. The

Table 1 - Rearing of *Palexorista laxa* (Curran) on diet based on the haemolymph extracted from *Manduca sexta* (L.)

Medium	Stage reared	Results				
		Treatment	Stage reached	N° %	Develop. days	Wgt (mg)
1) HEM from last instar larvae in Microtiter Plates: 5, 10, 20 µl per well, 1 maggot per well.	1st instar after 24 hrs in host	5 µl	3rd	1 10	—	—
		10 µl	3rd	4 40	—	—
(10 1st instar maggots per treatment)			Pupa	1 10	8	—
		20 µl		No development		
2) HEM from last instar larvae in small glass tubes. Two treatments:	1st instar after 24 hrs in host					
a) 10 µl hem per maggot (46 1st instar maggots)		10 µl HEM	2nd	13 28.3	—	—
b) 10 µl hem + 0.26% sterile soy flour per maggot (29 1st instar maggots)		10 µl HEM+0,26% soy flour	2nd	7 24.1	—	—
3) HEM from last instar larvae + soy residue in diverse %: 10, 15, 20, 25% in petri dishes (∅ 5 cm h 1.2 cm).	1st instar after 4-5 hrs in host					
(25 1st instar maggots per treatment divided into groups of 4 or 5)		20% soy residue	3rd	9 37.5	—	—
			Pupa	5 20.8	9.2	—
			Adul.	2 8.3	18	—
		25% soy residue	3rd	6 26.1	—	—
			Pupa	5 21.8	9.6	—
			Adul.	3 13.1	20	
4) HEM from last instar larvae + soy residue (20-25%) in wells in Micro-titer plates; 1 maggot per well (34 1st maggots)	1st instar after 4-5 hrs in host		3rd	24 75		
			Pupa	21 65.6	8.6	10.3

tachinid *Phryxe caudata* Rond. developed poorly *in vitro* when the free amino acid concentration of the artificial medium was identical to that of the host's haemolymph (Grenier *et al.* 1974). Development was much better when the concentration of free amino acids was increased in the artificial diet (Grenier *et al.* 1975). It is interesting that *P. laxa* developed on a haemolymph based diet from a species (*M. sexta*) that it probably would not encounter in nature. The reason that the adult yields were not higher than 13.1% may be due to the lower than optimal free amino acid concentrations of haemolymph or to the high fiber content of the diet. The fiber levels of the artificial diet are much higher than would be encountered in a living host and may adversely affect the maggots' digestive processes.

The development time of larval and pupal stages was recorded in tests 3 and 4. In the third test (tab. 2) average larval development time ranged from 9.2 (on 20% SR) to 9.6 (on 25% SR) days and was longer than the 6 days reported for *P. laxa* on *H. zea* held at the same temperature by Jackson *et al.*, 1976. For the pupal stage the same authors reported an average of about 9.4 days as compared to the 8.8 days on 20% SR and the 10.4 days for that on 25% SR. Development for the larvae was slower *in vitro* than *in vivo* and the effect on the pupal stage was dependent on the SR concentration. In the fourth test larval development was slightly faster (8.6 days) than in the third test. Here we also had very good yields of pupae (65.6%) and the pupal weights (10.3 mg) were roughly one fourth those of pupae from the host *H. zea*.

These findings show for the first time that lepidopteran haemolymph is a suitable nutritional medium for the whole larval development of a tachinid and this parallels studies of numerous Hymenoptera, whether they be oophagus, such as *Trichogramma* spp. (Hoffmann *et al.*, 1975; Xie *et al.*, 1986), and *Tetrastichus schoenobii* Ferriere (Ding *et al.*, 1980) or pupiphagous such as *Pteromalus puparum* L. (Bouletreau, 1968; Hoffmann *et al.* 1973). This is the first report that a species of tachinid has been reared to the adult stage on an artificial diet not containing agarose or agar. Much of the success of rearing *P. laxa in vitro* is

Table 2 - Duration of the developmental stages of *P. laxa* in the bollworm (from Jackson *et al.*, 1976) and *in vitro* on haemolymph-soy residue (SR) medium at 25°C temperature.

	Time required for development (days) of		
	Egg+larval stages	Pupal stage	Egg to adult
In bollworm (1)	6.00	9.4	15.4
In vitro (Third group)			
(20% S.R.)	9.2 (2)	8.8	18.00
(25% S.R.)	9.6 (2)	10.4	20.00

(1) Parasitization occurred at late 4th, 5th and early 6th larval instar.
(2) The time is calculated from 4-5 hours after hatching.

due to the long everted spiracles which the maggots place above the surface of the liquid medium. Our success with SR containing diets indicates that species of tachinids with everted spiracles may be good candidates for economical large-scale production on artificial diets containing substitutes for expensive agar and agarose.

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SUMMARY

The authors report the results of *in vitro* rearing of maggots of the tachinid *Palexorista laxa* (Curran) using a medium based on *Manduca sexta* L. haemolymph. Soy residue, added to the medium, thickened the artificial diet and served as a physical support for the maggots.

Allevamento *in vitro* di *Palexorista laxa* (Curran) (Diptera: Tachinidae), su diete a base di emolinfa

RIASSUNTO

Gli autori riportano i risultati dei primi tentativi dell'allevamento *in vitro* degli stadi larvali del dittero tachinide *Palexorista laxa* (Curran). I substrati saggiati sono stati tutti a base di emolinfa estratta da larve di *Manduca sexta* L., con aggiunta di residuo della lavorazione della soia quale elemento di supporto fisico per le larve del parassita.

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