

RADIOGRAPHIC DETECTION OF PUPAL PARASITES OF THE LARCH CASEBEARER, *COLEOPHORA LARICELLA* (LEPIDOPTERA: COLEOPHORIDAE)¹

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ABSTRACT

A radiographic technique was used to detect hymenopterous parasites of the larch casebearer, *Coleophora laricella* (Hübner), from Idaho. Pupal characteristics of *Bracon pygmaeus* Provancher, *Spilochalcis albifrons* (Walsh), *Chrysocharis laricinellae* (Ratzeburg), *Diadocerus nearcticus* Yoshimoto, *Tetrastichus* sp., and *Mesopolobus verditer* (Norton) were examined from radiographs and compared. A key was constructed to identify pupae of these parasites.

INTRODUCTION

Within 30 yrs, the larch casebearer, *Coleophora laricella* (Hübner) (Lepidoptera: Coleophoridae), has infested many stands of western larch, *Larix occidentalis* Nuttall, in the Pacific Northwest. In seeking to control its high populations, native hymenopterous parasites have been surveyed (Andrews and Geistlinger 1969; Bousfield and Lood 1973; Miller and Finlayson 1974, 1977) and imported parasites released (Denton 1972, Ryan and Denton 1973, Ryan et al. 1977). Typically, analyzing parasitism by collecting hosts and rearing-out the parasites is labor intense, demands much laboratory space, and is time consuming. However, radiographic methods are more efficient because less labor is needed and they can often be used in the field.

Concealed parasites of forest insect pests have been identified by using radiography (Holling 1958, Yates 1967, Thornbury and Denton 1972). In studies of parasites of the larch casebearer, Amman and Tunnock (1971) detected *Agathis pumila* (Ratzeburg) (Hymenoptera: Braconidae) pupae within pupal cases of the host. Similarly, Eidmann (1962) followed the development of the European parasites *Chrysocharis boops* Thompson (Hymenoptera: Eulophidae) and *Cirrospilus pictus* (Nees) (Hymenoptera: Eulophidae) in casebearer larvae. This paper reports an efficient, non-destructive method of detecting the important parasites of the larch casebearer in the Pacific Northwest.

MATERIAL AND METHODS

Pupating larch casebearers were collected June 26-27, 1975 near Potlach, Idaho, in Latah Co., and July 3, 1975 near Priest River, Idaho, in Bonner Co. About 1,500 pupae were isolated

and radiographed 2 wks and one month after collection by using a Faxitron® X-ray machine with an output of 10-110 kv and continuous 3 ma. Casebearers were arranged in a grid on a black vinyl sleeve covering a sheet of double-coated Kodak® Type R Industrial film. Exposures used in previous detection studies of parasites of the casebearer were 14 kv, 10 ma for 1.6 sec with a target distance of 25 cm (Eidman 1962) and 5 kv, 3 ma for 4 min at 50.8 cm (Amman and Tunnock 1971). In this study, the best results were at 15 kv, 3 ma for 4 min at 45 cm target distance.

After exposure, pupae were maintained at 21-26°C in separate vials. Emerging parasites were identified to species and sex, and their radiographs as immatures were examined for distinguishing characteristics.

RESULTS AND DISCUSSION

By inspection of the radiographs, pupae of nonparasitized larch casebearers (Fig. 1) were easily separated from ones containing parasites. Although their activity was obvious, most parasitic larvae were nonidentifiable because they were without detectable characteristics or had moved during exposure. However, pupae of the parasites had consistent identifying characteristics like body length and shape, position of the head, structure of the legs, and relation between thorax and abdomen. Of the larch casebearer parasites in Idaho, these six are the most common (Bousfield and Lood 1973).

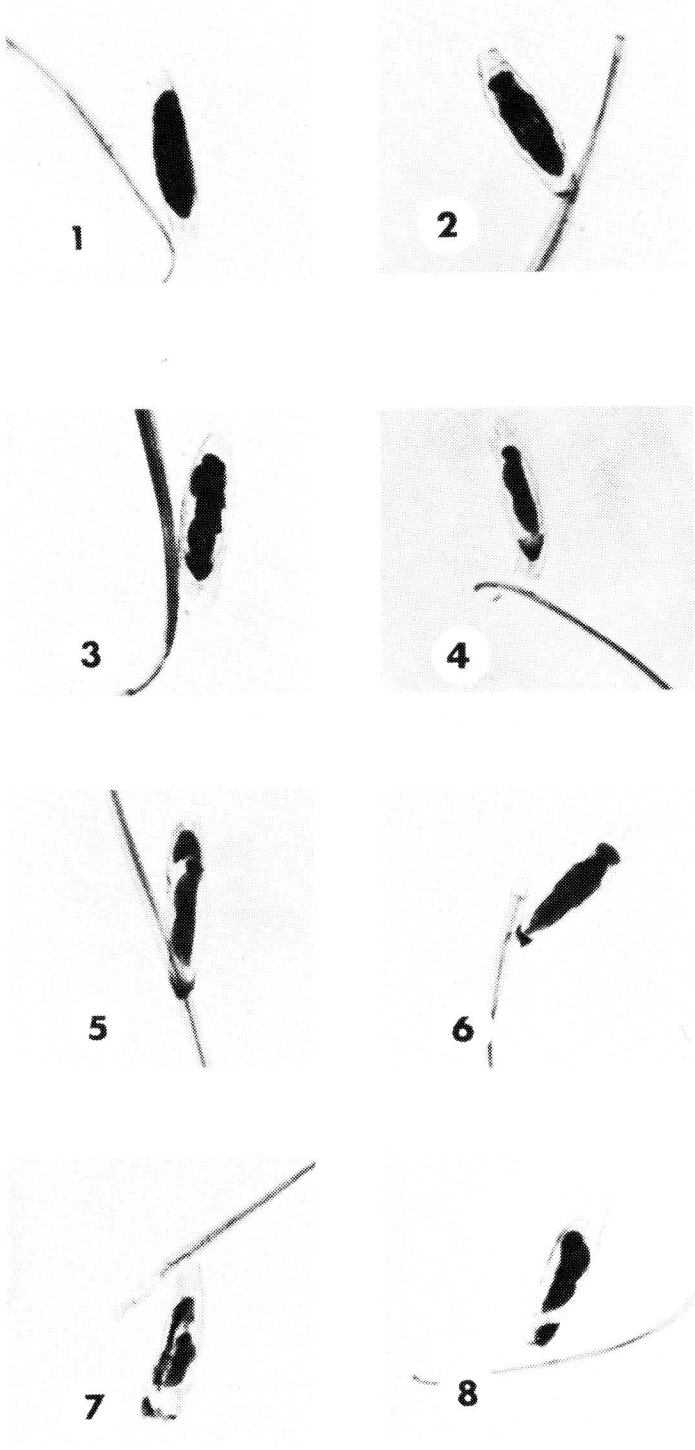
The pupa of *Bracon pygmaeus* Provancher (Hymenoptera: Braconidae) (Fig. 2) had a long abdomen with a noticeable petiole and was the only one having a head with anterior "projections", actually antennal bases. The radiograph of the similar *Agathis pumila* was described by Amman and Tunnock (1971).

The enlarge metafemora distinguished the pupa of *Spilochalcis albifrons* (Walsh) (Hymenoptera: Chalcididae) (Fig. 3). The pupal head was always toward the caudal region of the casebearer, and the meconium, the larval waste

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Figs. 1-8 — Radiographs on Kodak® Type R Industrial film (8.5X) of a normal pupa of the larch casebearer, *Coleophora laricella*, and pupae of parasitoids of the larch casebearer. 1, Larch casebearer. 2, *Bracon pygmaeus*. 3, *Spilochalcis albifrons*. 4, *Chrysocharis laricinellae*. 5, *Diadromocerus nearcticus*. 6, *Tetrastichus* sp. 7, Male *Mesopolobus verditer*. 8, Female *M. verditer*.

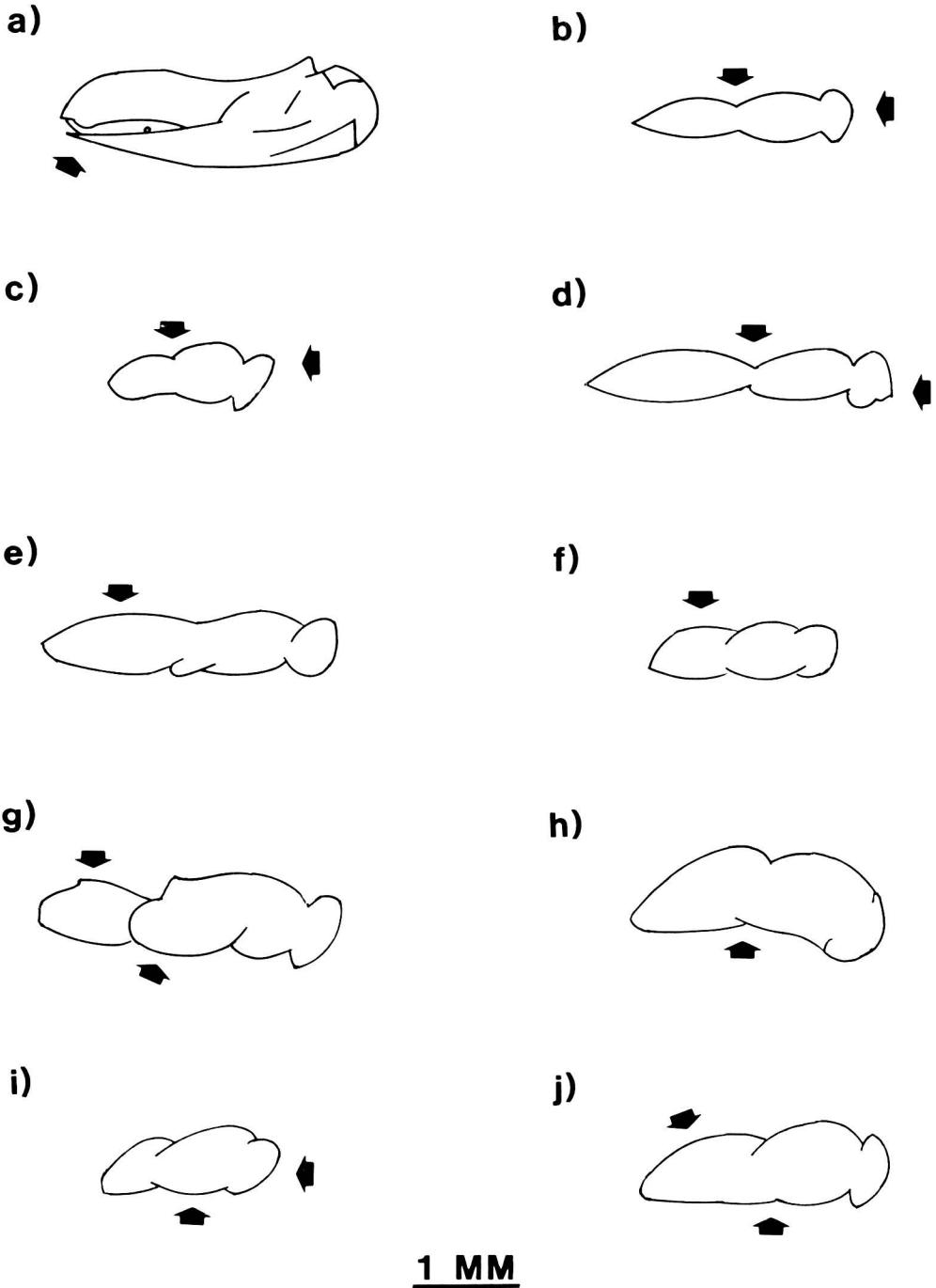


Fig. 9 — Diagrams from radiographs of pupae of six major parasites of the larch casebearer, *Coleophora laricella* (lateral view); a, normal larch casebearer pupa; b, generalized elongate pupa; c, generalized robust pupa; d, *Bracon pygmaeus*; e, *Dicladocerus nearcticus*; f, *Tetrastichus* sp.; g, *Spilochalcis albifrons*; h, female *Mesopolobus verditer*; i, male *Mesopolobus verditer*; j, *Chrysocharis laricinellae*.

of the parasite, was deposited directly below the pupa of the parasite. Oddly, mostly males emerged from larch casebearers (Hansen 1980). Although *S. albifrons* is often hyperparasitic, the radiographs never showed secondary parasitism.

The eulophid parasites were similar to each other. The pupa of *Chrysocharis laricinellae* (Ratzeburg) (Fig. 4) was the broadest or most robust, having the head appressed to the thorax. In the pupa of *Dicladocerus nearcticus* Yoshimoto, (Fig. 5) the abdomen was noticeably larger than the thorax. This parasite was one of the first to emerge, mostly as females, and often in areas where *S. albifrons* was absent (Hansen 1980). The pupa of *Tetrastichus* sp. (Fig. 6) was smaller than *D. nearcticus* and the abdomen was the same size as the thorax; like *C. laricinellae*, both sexes emerge from casebearers.

The pupa of the pteromalid *Mesopolobus verditer* (Norton) (Figs. 7,8) had a distinctive "bent" form. Females were similar to males but larger, being greater than 2 mm in length. This species was very common in some areas.

By comparing recognizable pupal characteristics from the radiographs, a key to identifying the parasites was constructed. Because the characters were often obscured dorsally, the key refers only to the lateral position of the pupa.

KEY TO X-RAY IMAGES OF PUPAE OF SIX MAJOR LARCH CASEBEARER PARASITES

- 1 Wings almost as long as body, metathoracic legs terminate near posterior apex of body; lepidopterous pupa (Fig. 9a) *Coleophora laricella*
- 1' Wings short and near middle of body, legs terminate well before posterior apex of body; hymenopterous pupa 2
- 2(1') Head in same plane as body and separate from thorax; body elongate and narrow,

- sides parallel; often constriction between abdomen and thorax (Fig. 9b) 3
- 2' Head slightly slanted forward and appressed to thorax; body robust, sides more convex; abdomen and thorax not distinctly separate (Fig. 9c) 5
- 3(2) Base of antennae projected forwards beyond head; abdomen twice as long as thorax, narrow constriction between abdomen and thorax; large (2.5 mm); not common (Fig. 9d) . *Bracon pygmaeus*
- 3' No projection in front of head; abdomen less than twice as long as thorax, abdomen and thorax broadly joined; body length variable 4
- 4(3') Abdomen larger than thorax; body length 2.5 mm (Fig. 9e) *Dicladocerus nearcticus*
- 4' Abdomen about the same size as thorax; body length less than 2.5 mm (Fig. 9f) *Tetrastichus* sp.
- 5(2') Metathoracic femur large; abdomen smaller than thorax; large (2.5 mm) (Fig. 9g) *Spilochalcis albifrons*
- 5' Metathoracic femur small; abdomen usually larger than thorax; body length variable 6
- 6(5') Body length greater than 2 mm; body curved into a "bent" position (Fig. 9h) *Mesopolobus verditer*♀
- 6' Body length less than or equal to 2 mm; body position variable 7
- 7(6') Body in a "bent" position, head clearly tilted; thorax may be longer than abdomen; body length ca. 1.5 mm or less (Fig. 9i) *Mesopolobus verditer*♂
- 7' Body more horizontal; abdomen usually longer than thorax; body length ca. 2 mm (Fig. 9j) ... *Chrysocharis laricinellae*

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THE PROPORTION OF IMMATURE STAGES OF THE ROCKY MOUNTAIN WOOD TICK (*DERMACENTOR ANDERSONI*) FEEDING ON ARTIFICIALLY INFESTED CATTLE

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ABSTRACT

Only 2% of *D. andersoni* larvae engorged when confined in sleeves over clipped areas on or near the tails of two heifers, but percentages were higher in some sleeves. About 13% of nymphs fed when placed in sleeves near the withers of two other heifers. These yields are within 10 percentage points of those from two usual laboratory hosts (rabbits and white mice), which suggests that cattle should be examined for larvae and nymphs in the field, since the numbers feeding on them may not always be negligible in relation to disease transmission and maintenance of tick populations.

INTRODUCTION

It is generally accepted that, in nature, larvae and nymphs of *Dermacentor andersoni* attach to small and medium size rodents and lagomorphs, whereas the adults feed on medium to large mammals (Cooley 1932, Gregson 1956). The only hosts on which all three instars commonly feed are porcupines (*Erethizon dorsatum*), jackrabbits (*Lepus townsendi*), and marmots (*Marmota* spp., especially *M. flaviventris*) (Gregson 1956, Wilkinson 1970).

Wilkinson (1970) found an engorged nymph in a batch of adult *D. andersoni* from a mule deer (*Odocoileus hemionus*), raising the question of the degree of infestation of larger mammals by immature *D. andersoni* in nature. In studies on transmission of anaplasmosis, larvae and nymphs have been placed on cattle (Rees 1934, Anthony and Roby 1966) and have fed, but no indication was given of the proportion

feeding, which might provide a guide to the probability of infestations in nature. Rees (1934) confined the ticks in bags on the scrota of bulls; Anthony and Roby (1966) used bags taped near the base of the tail of calves, a location that was inconveniently wet with urine or feces with our heifers. In the present paper, the proportions feeding in sleeves on cattle are compared with some routine infestations of laboratory mice and rabbits in which the larvae and nymphs were liberated on the hosts without the use of sleeves or capsules. Rabbits are commonly used for laboratory cultures of Rocky Mountain wood ticks (Gregson, 1966), and Kohls (1937) stated that about 19% of larvae and 30% of nymphs fed and were recovered in mass cultures of *D. andersoni* on laboratory rabbits.

The aim of the work described here was to elucidate the proportion of immature stages

feeding on cattle after artificial infestation since, if very few or none fed, there would be little incentive to attempt the difficult task of examining range cattle for feeding larvae or nymphs. The numbers may not be commonly important as a contribution to tick populations, but could be important in clarifying the epizootology of anaplasmosis (Peterson 1973). This disease has been detected serologically in cattle in Idaho near the British Columbia border (Long *et al.* 1974).

METHODS

Cattle Larvae

Engorged female ticks collected from cattle in British Columbia in April 1980 were kept at about 5°C until 23 May, after which they were incubated in individual vials at 25°C over saturated KNO₃ (95% R.H.). Larvae emerging from their eggs were transferred to 10°C on 3 July for storage until 31 October.

On the day before infestation, organdy sleeves were fixed with contact cement to closely clipped circular areas of skin, enclosing a central area of about 5 cm in diameter on which the hair had been clipped to about 1.5 cm long. One such sleeve was placed on each side of the tail-root of each of two yearling Hereford heifers, stanchioned in a barn. In addition, one sleeve was placed around the tail, just above the tail switch, with an opening in the side of the sleeve to insert the larvae. The progeny of one female tick was inserted in each sleeve, i.e., about 4,200 larvae per sleeve. This is based on a 500-mg (visual estimate) female tick yielding 5,200 eggs (Wilkinson 1968, table VII) with 90% hatch and 90% survival. Ambient temperatures in the barn ranged from 12 to 18°C. After insertion of the larvae on 31 October, the sleeves were closed with elastic bands and examined for fed larvae 3, 4, 5, 6, and 7 days later. The fed larvae were removed by suction, or using a moist fine paint brush, and placed in a desiccator over saturated NaCl (75% R.H.) at 25°C, to check viability of the next instar.

Nymphs

The nymphs used had emerged from stock larvae that had fed on laboratory rabbits and were then kept at 25°C over NaCl until ecdysis was completed. The nymphs were then stored at 10°C over saturated KNO₃ for 1 month. Infesting procedures were similar to those for larvae except that one sleeve was placed on the top and one on the left or right side of the withers of each of two heifers; about 300 nymphs were placed in each sleeve on 12 March 1980.

The sleeves were checked for engorged nymphs 5, 6, 7, and 8 days after infestation. The fed nymphs were removed from the cattle and stored in the same way as the fed larvae.

Mice

Mice were infested by shaking or extruding the larvae from tubes, or modified plastic syringes in which the larvae hatched, onto the heads of mice fitted with antigrooming collars. The mice were then placed in individual cages surrounded with chalk barriers (Wilkinson 1964) to retain the fed larvae. Each mouse received unfed larvae derived from 20- or 40-mg egg batches (ca. 330 or 660 larvae).

Rabbits

Rabbits were infested with an average of 1,280 or 1,720 nymphs/rabbit and then caged individually over a table fitted with a raised water channel to retain the engorged nymphs.

RESULTS

Based on the estimate of about 4,200 viable unfed larvae per sleeve, the 479 fed larvae in Table 1 represent a yield of 2%. Omitting 8,400 larvae applied near the tail switches, the yield was 3% for the two animals, or as high as 7% for the highest yielding sleeve. This compares with yields of 11 to 31% recorded from four tests with batches of four or eight white mice. The highest yields on cattle were on the 4th and 5th days after infestation whereas, with the mice, the peak yield was on the 3rd or 4th days when the ambient temperature was about 27 or 20°C respectively.

The yield of fed nymphs from the cattle was 13% (Table 2) whereas, in two tests involving five or six rabbits each in the vivarium, it was 13 and 18%. The number of nymphs from the cattle peaked on the 6th day (Table 2) whereas, from the rabbits, the numbers peaked on the 4th and 5th days with ambient temperatures of about 23 to 28° or 20°C respectively.

DISCUSSION

The larvae and nymphs in the sleeves on cattle were unlikely to have been much assisted, relative to unclipped cattle, by the clipping of the hair inside the sleeves to about 1.5 cm. Similar-sized larvae and nymphs of the southern cattle tick and the winter tick normally feed on both long- and short-haired ungulates.

In the present work, nymphs were applied to the cattle on the withers because this is an area naturally infested by adults (Wilkinson 1972); it is a good site for sleeves because cattle rarely rub them off. The tail region was used for larvae because Anthony and Roby (1966) had succeeded in feeding larvae in that area, and it was considered that the short mouthparts of the larvae might limit the areas on which they could feed to engorgement.

The fed larvae and nymphs from cattle, mice, and rabbits produced normal numbers of the next instars when kept as stated earlier.

TABLE 1. Yield, by site and day of collection, of detached¹ engorged larvae after infestation of two heifers (A and B) on 31 October 1980 with about 4,200 unfed larvae per site.

Days after infestation	Animal	Site of infestation			Total larvae for day
		Near tail switch	Pelvic area (tail-root)		
			Left side	Right side	
3	A	0	0	1	
	B	0	0	0	1
4	A	3	17	114	
	B	0	40	29	203
5	A	0	57	113	
	B	0	33	2	205
6	A	0	10	9	
	B	0	3	1	23
7	A	0	1	43	
	B	0	3	0	47
TOTAL		3	164	312	479 ²

¹ Values for day 7 include some fed larvae that had not detached.

² Equal to 2% of larvae applied.

The yield of larvae and nymphs from cattle were considered sufficiently comparable to those from the favorable hosts (i.e., mice and rabbits) to suggest that cattle on infested range should be closely examined for immature stages at appropriate times of the year. Host-seeking larvae are most abundant in British Columbia grasslands in July and nymphs in April-May and August (Wilkinson 1968, 1979).

Range cattle in areas in British Columbia most infested with *D. andersoni* are not normally handled or mustered except in the winter feedlots or in late March-early April (for calving and acaricide treatment), or in June (for branding and movement to forest grazings), and in September for weaning and return to lower altitudes. Special arrangements would have to be made to examine the cattle for larvae in July, and in many areas the cattle would not be in contact with the tick foci (e.g., rocky outcrops in the wheatgrass/ Ponderosa pine zone) except in April-June. In southern

Alberta, cattle enter the tick-infested forest grazings of the eastern foothills of the Rocky Mountains in early June and could advantageously be examined for larvae in July if a muster could be arranged. British Columbia cattle could possibly be examined for late-occurring 'spring' nymphs in June, during branding. Alternatively, cattle not treated with acaricides might be examined for nymphs in late April or May, involving a special muster or examining animals that have become paralyzed by the adult ticks. The length of the haircoat and the necessity to examine the cattle all over could make the search for small unfed immature ticks difficult. In both British Columbia and Alberta, few nymphs and larvae would be available to parasitize cattle in September.

The observations reported here on yearling heifers, infested on the withers and tail-root, supplement the observations of Rees (1934) and Anthony and Roby (1966), who used the scrota of bulls or calves' tails as infestation sites.

TABLE 2. Yield, by site and day of collection, of detached¹ engorged nymphs after infestation of two heifers (C and D) on 12 March 1980 with about 300 nymphs per site.

Days after infestation	Animal	Site of infestation		Total larvae for day
		Withers		
		Top	Side	
5	C	12	2	24
	D	2	8	
6	C	62	1	83
	D	3	17	
7	C	18	3	25
	D	0	4	
8	C	3	9	20
	D	0	8	
TOTAL		100	52	152 ²

¹Values for day 8 include some fed nymphs that had not detached.

²Equal to 13% of nymphs applied.

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A SIMPLE AND INEXPENSIVE STATIC SPREADING BOARD FOR MICROLEPIDOPTERA

DAVID R. GILLESPIE

A static spreading board for Microlepidoptera, similar in principle to that described by Martin (1977 pp92-93), but more easily constructed from readily available materials, was devised to save money. It considerably reduced the time and damage involved in handling when compared with a standard, wooden, non-static spreading board.

The board is of styrofoam, 75 mm wide x 280 mm long x 24 mm thick with a longitudinal centre groove (fig. 1). A layer of inexpensive sandwich wrap is fastened to the surface with a silicon sealant and is forced into the groove with a pencil or dowel. Styrofoam sheets of the appropriate thickness are readily available because they are commonly used for insulation.

The dimensions of the groove and the board may be varied to fit almost any need. A groove 10 mm wide and 5 mm deep was suitable for large microlepidoptera but a groove 5 mm wide and 5 mm deep was better for small species.

To use the board, first statically charge it by rubbing with a dry cloth. The wing bases of the

pinned moth should be even with the surface of the board. Blowing from behind the wings forces them to an extended position where they are held in place by the static charge. They are then fastened with glassine paper strips held in place by pins. The thorax of the specimen should be 10 to 11 mm below the head of the mounting pin.

Boards of this design were used in 1979 and 1980 to mount large numbers of reared Lepidoptera, and were especially suitable for Tortricidae.

The sandwich wrap did not remove many scales from the undersides of wings. It is essential that all specimens be freshly killed, as the static system does not work with rehydrated or stiffened specimens. One minor drawback is that after the board has been used many times, the sandwich wrap becomes so perforated that it no longer functions as a static surface, and may actually abrade the wing surfaces. When this happens the layer can simply be replaced.

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Martin, J. E. H. 1977. *The Insects and Arachnids of Canada. Part 1. Collecting, Preparing and Preserving Insects, Mites and Spiders.* Agriculture Canada Publication 1643.

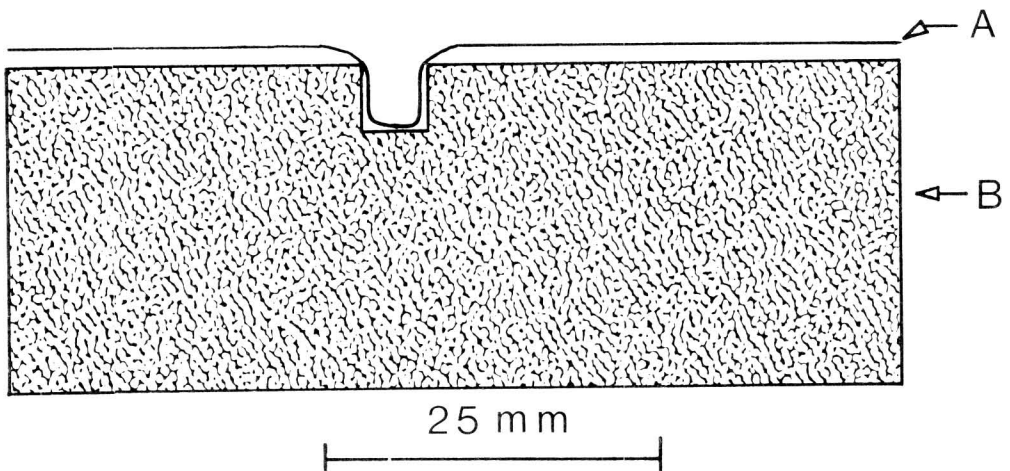


Fig. 1. Cross section of styrofoam spreading board. A, sandwich wrap overlay; B, styrofoam.