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Egg and Larval Development in the Woodwasp, *Sirex noctilio* F.

J. L. Madden

Faculty of Agricultural Science, University of Tasmania, G.P.O. Box 252C, Hobart, Tas. 7001.

Abstract

Development and survival of the immature stages of *S. noctilio* are dependent on active growth of the symbiotic fungus, *Amylostereum areolatum* (Fr.) Boidin. Suppression of fungal growth by excess moisture or low temperature prolonged embryogenesis, so that eclosion might be delayed until 300 days post oviposition in the base of some attacked trees, compared to 15–30 days within the warmer and drier tops of the same trees. Enhanced respiratory activity of the fungus may trigger egg development, for eggs exposed to carbon dioxide matured more rapidly than those exposed to air. Pure oxygen and nitrogen were toxic to eggs, and surface treatment with dilute ethanol delayed development. The development of the *Sirex* egg is described for the first time.

The larvae fed on fungus-infested wood; the conversion ratio of larval weight change to weight of wood displaced per instar decreased with successive instars. This reduction resulted from the progressive decline of fungal food substrate and overall moisture content. The ultimate size of *S. noctilio* adults was determined by conditions which favoured fungal growth, and the fungus transported nitrogen through the wood to feeding larvae.

Introduction

The eggs of the woodwasp *S. noctilio* F. are deposited in shafts drilled by the female through the bark and into the xylem or outer sapwood of the host tree, *Pinus radiata* D. Don. The frequency of shafts and, in turn, eggs at a drilling site depends on the physiological status of that site, so that more eggs are deposited as the tree progressively succumbs to attack (Madden 1968, 1974). The arthrospores of the symbiotic fungus *Amylostereum areolatum* (Fr.) Boidin are inoculated during drilling activity (Coutts and Dolezal 1969).

Fungal development precedes eclosion and larvae develop and grow by feeding through fungus-infested wood. Clark (1933) reported the presence of mycelial fragments within *S. noctilio* larvae but this claim was disputed by Boros (1968) and Morgan (1968). The latter authors believed that digestion was primarily extra-intestinal and that no woody material was ingested. Feeding activity results in galleries filled with compacted frass. Rafes (1960, 1961) stated that the shape of the gallery of *Paururus* (= *Sirex*) *noctilio* in Russia could take any one of four distinct forms, viz. looped, half-looped, reversed or kinked (cf. Fig. 1), and concluded that the direction and length of each section of the gallery was related to distinctive stages in larval development, that turning facilitated the shedding of exuviae, and that unfavourable environmental conditions could induce deviations from the optimal path of developing larvae.

These findings are compared with the development of *S. noctilio* eggs and larvae under the milder climatic conditions of Tasmania.

Methods and Materials

All experiments were conducted at the Field Station of the Division of Entomology, CSIRO, at Llanherne, Tas., utilizing both field and laboratory *Sirex*-infested material.

The analysis of the effects of temperature on the development of *Sirex* eggs, complete insect development and fungal growth was based on the concept of the thermal constant and expressed in units of day-degrees (Andrewartha and Birch 1954).

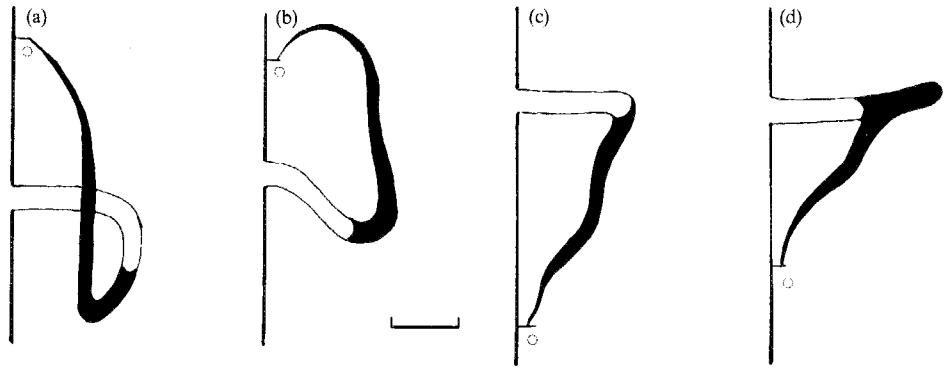

Fig. 1. Types of siricid gallery: (*a*) looped; (*b*) half looped; (*c*) kinked; (*d*) reversed. ○ Oviposition site. Scale line, 1 cm. After Rafes (1960).

Experimentation: Methods and Results

Development of S. noctilio *Eggs*

(i) *Methods*

Fresh billets of *P. radiata*, 2 m long, were exposed to ovigerous *S. noctilio* females for 24 h in continuously illuminated indoor cubicles at a temperature of 22 ± 2°C. Sections of these billets were subsequently end-coated with grafting mastic (Shell 2M), to avoid excessive desiccation, and held at different temperatures. Drilled sites were sampled daily by removing 2·5-cm^3 blocks with a knife-edge chisel. The shafts were dissected and eggs transferred to sterile 5% agar plates. Alternatively, females were permitted to drill through pieces of thick bark held in contact with moist blotting paper lying on a debarked billet. Large numbers of eggs were harvested from the paper surface as the thick bark prevented deposition into the wood. Samples of these eggs were transferred on a flamed needle either directly or through washes of streptomycin sulphate or 70% ethanol, and subsequent washes of sterile water, to sterile agar plates. Treatment with antimicrobials was found necessary to prevent bacterial contamination.

Plates plus eggs were incubated at different temperatures and examined daily beneath a binocular microscope by transmitted light; drawings were made with the aid of a camera lucida to record progressive changes in the appearance of eggs. Subsequent examination of the drawings permitted an appreciation of embryogenesis.

The effects of gas levels on development were evaluated by confining egg plates in closed metal containers which were flushed out with either nitrogen, oxygen or carbon dioxide after each daily examination. In all tests a minimum of 30 eggs were observed.

(ii) *Results*

Development of eggs depended primarily on temperature and the minimum time to eclosion was 8 days at 30°C. However, this temperature resulted in 18·5% mortality (n = 64 eggs). The optimal period was 10–12 days at 25°C, and below

this temperature the time taken for development became increasingly prolonged. At 10°C approximately 64 days elapsed before hatching. The threshold temperature was 6·2°C and the thermal constant averaged 186·3 day-degrees (Fig. 2). Embryogenesis was shown by contraction of the germ band, alteration in the appearance of the yolk, the development of segmentation and, ultimately, by movement by the embryo. Such changes are depicted in Fig. 3 and described in Table 1.

Eggs dissected from drills and then exposed to normal air developed to hatching in 10–12 days at 25°C. However, eggs collected from blotting paper and which had spent no time within a shaft required 13–14 days incubation before hatching. Exposure of eggs to carbon dioxide accelerated development by 1–2 and 4 days for dissected and 'free' eggs respectively. Exposure to nitrogen and oxygen promoted marked changes in egg appearance and was lethal. Nitrogen resulted in extensive tanning of the chorion after 2–3 days at 25°C; oxygen completely inhibited development, and produced gross distortion in egg shape and sometimes rupture.

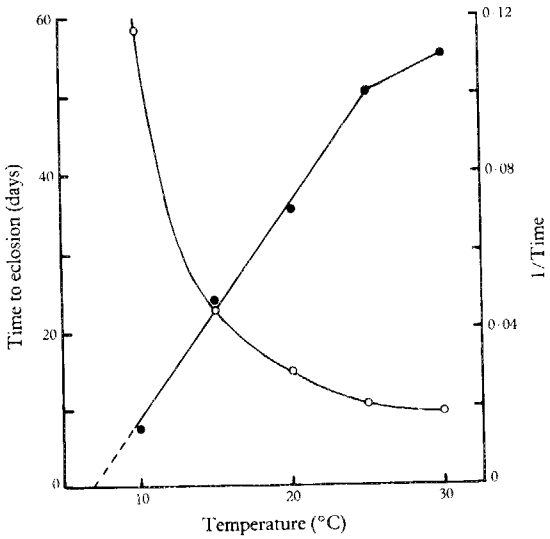

Fig. 2. Time to eclosion of *S. noctilio* eggs incubated at different temperatures (○), and resultant rates of development (●).

Differences in development were also observed between the antimicrobial treatments. Streptomycin sulphate did not affect normal development, whereas ethanol treatment followed by sterile water washes resulted in a slight tanning and puckering of the chorion; these eggs required 35–40 days to complete their development at 20°C, compared to 14–16 days for dissected eggs and those treated with antibiotic.

Respiration of Wood Blocks containing S. noctilio *Eggs and the Symbiotic Fungi*

(i) *Methods*

Wood blocks 2·5 cm^3, containing oviposition drills of known age, were removed at 4-day intervals from billets held at 25°C, uniformly split, weighed and enclosed in Warburg manometer flasks. A complementary series of blocks with no drills was removed from the same billets and treated in the same way. The flasks were equilibrated for 20 min in a waterbath held at 27 ± 1°C, and respiratory activity assessed during the following 60 min. Eggs were dissected from shafts after each run and their

development noted. The wood was dried at 96°C and moisture contents assessed on an oven-dry-weight (ODW) basis.

(ii) *Results*

The respiration of wood blocks containing oviposition drills was sustained at a relatively constant rate for 10 days after oviposition, and then declined. In contrast, the respiration of drill-free wood blocks was initially low but increased to

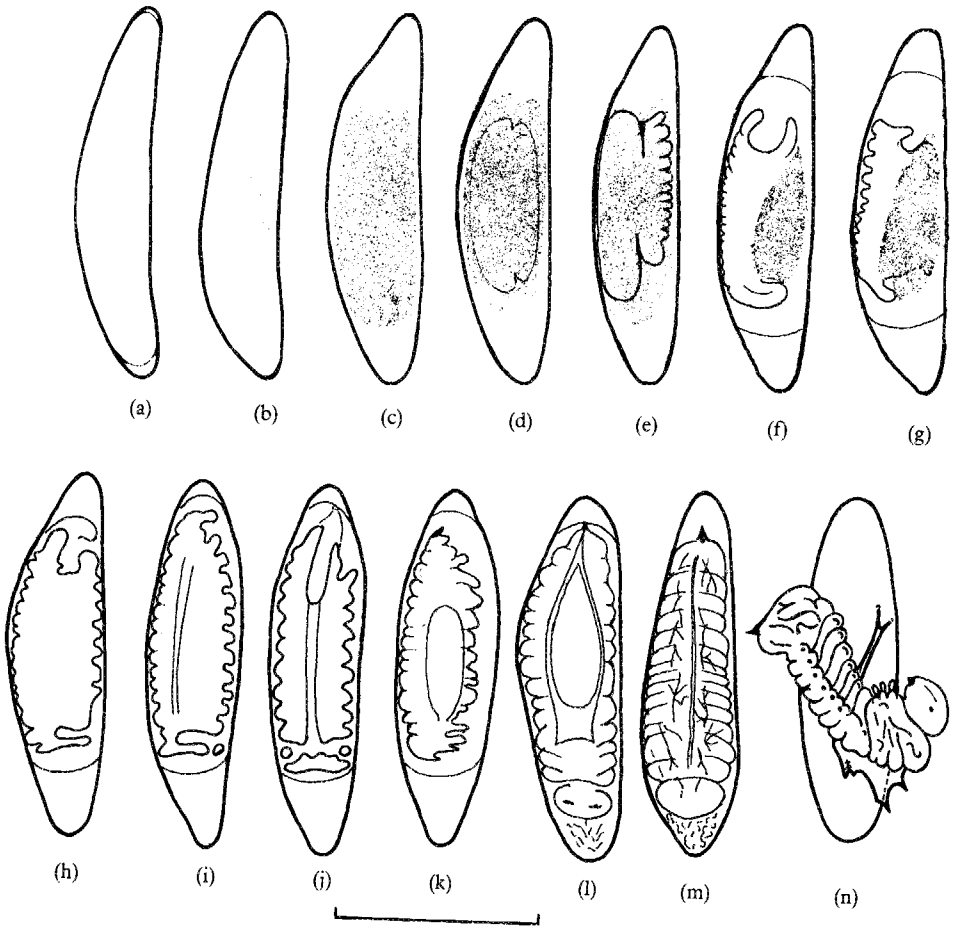

Fig. 3. The appearance of *S. noctilio* eggs, incubated at 20°C on 5% agar, at different stages of development. For explanation of stages, see Table 1. Scale line, 1 mm.

a rate comparable to that of the drill blocks by day 10, and then also declined. Changes in moisture content indicated a net movement of water toward the oviposition drills; changes in both respiration and moisture content differences are shown in Fig. 4.

Egg eclosion coincided with the onset of the decline in the respiration of both the test and control blocks, i.e. about 10 days.

Table 1. Stages in the development of *S. noctilio* eggs incubated at 20°C on 5% agar

Time (days)	Aspect	Apparent stage of development	Fig. 3 reference
0–1	Lateral	Newly laid egg, translucent with germ band starting to form	*a*
1–2	Lateral	Progressive condensation of germ band	*b*
2–3	Lateral	Yolk becoming more frothlike in appearance	*c*
4	Lateral	Initiation of segmentation, formation of caudal and cephalic lobes causing slight invagination of yolk	*d*
5–6	Lateral	Segmentation apparent; embryo rises in yolk	*e*
6–7	Lateral	Progressive envelopment of yolk by embryo	*f, g*
8	Lateral	Fully segmented embryo; cephalic region distinct	*h*
9	Lateral	Gnathal elements and mesenteric organization appear	*i*
11	Dorsal	Closure of dorsal wall; stomodeum and proctodeum evident	*j*
12	Lateral	Formation of head and mesenteron; dorsal spine pigmented;contraction of embryo within serosa	*k*
13	Ventral	Head complete, alimentary canal and mandibles evident; rapid increase in size through consumption of yolk remnants and pneumatization; embryo moving, chorion wrinkled	*l*
15	Dorsal	Fully formed embryo within serosa; tracheation apparent	*m*
16	Lateral	Eclosion of larvae after consumption of serosa and rupture of chorion	*n*

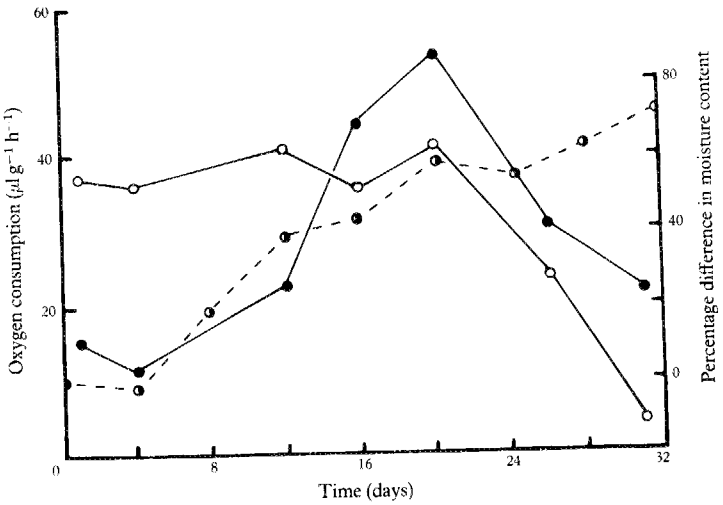

Fig. 4. Respiration of blocks of xylem with (○) and without (●) oviposition drills, and the percentage difference in moisture content (◑; test block minus control block).

Development of S. noctilio *within* P. radiata *Wood*

(i) *Methods*

P. radiata billets 1 m long were infested as above and incubated at different constant temperatures. Initial wood moisture contents were obtained from discs, cut in the preparation of the billets, which were then end-coated with grafting mastic. The billets were examined each week, and the time that the first emergents appeared, and the diameters of emergence holes, were recorded. The diameter of the emergence hole is equivalent to the width of the prothorax of the emergent; this measure (*pt. w.*) was utilized as the index of adult size (Madden 1974).

(ii) *Results*

The time for the first insects to emerge from billets of different initial moisture contents was comparatively uniform (± 1 week) although the size varied. Billets of high initial moisture (>180% ODW) yielded small adults (*pt. w.* 2·5–3·0 mm); large insects (*pt. w.* 5·5–6·4 mm) emerged from billets of moderately low initial moisture content (80–100% ODW).

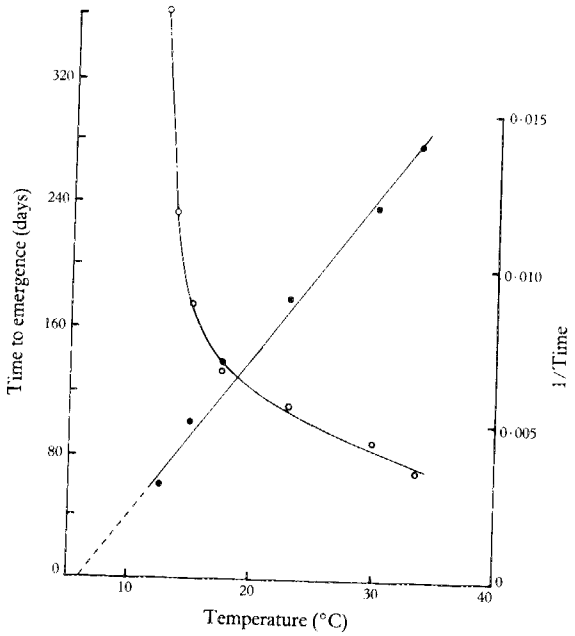

Fig. 5. Time from oviposition to first emergence of *S. noctilio* adults from billets incubated at different temperatures (○), and their overall rates of development (●).

Rates of development to the adult stage were also dependent on temperature; the minimum observed time for development was 8–9 weeks at 33·5°C, but mortality was high (≃60%). The thermal constant was of the order of 2500 day-degrees and the threshold temperature recorded was not significantly different to that observed for eggs, i.e. 6·8°C versus 6·2°C (Fig. 5).

Larval Feeding and Gallery Characteristics

(i) *Methods*

The general shape of larval galleries was examined in billets from different sources and treatments and of varying diameters (3·0–90·0 cm). Billets were split open and individual galleries fully dissected with scalpels and chisels. Half of each gallery was exposed longitudinally to reveal the exuviae embedded in the compact larval frass. The diameter of the gallery at the site of each moult and the

distance between consecutive moults were measured with vernier calipers or a micrometer eyepiece. These dimensions described a series of truncated cones and the volume of frass produced by each instar was calculated from the formula:

$$V_x = (L_x/3)A_x + \sqrt{(A_x \cdot A_{x-1})} + A_{x-1},$$

where V_x is the volume of frass produced by instar x, L_x is the length of gallery between exuviae $(x-1)$ and x, A_x is the cross-sectional area of gallery at exuviae at end of L_x, and A_{x-1} is the cross-sectional area of gallery at exuviae at beginning of L_x. Proceeding from smaller to larger sections, A_x became A_{x-1} for the following instar.

The bulk densities of wood and frass were determined by weighing cubes of oven-dried wood of known dimensions and frass from measured sections of galleries. These estimates were used to: (1) convert volumes of frass to weights of original wood displaced; (2) obtain a measure of the utilization of wood by fungus and insect.

The direction of larval galleries within standing trees was also recorded, to assess the influence of drying patterns on larval behaviour. Five trees approximately 15 m high were sampled at intervals of 1 m by removal of a 15-cm section which was split and the directions of galleries with respect to the original oviposition sites noted.

Currently infested billets were also split and the depth and diameter of the space occupied by larvae, together with larval head capsule widths, sex, and live and dry weights recorded. The sex of larvae was determined by the presence (in females) or absence (in males) of hypopleural organs, which carry the symbiotic fungus, between the first and second abdominal segments.

(ii) *Results*

The majority of galleries examined were of the half-looped pattern (Fig. 1). The dimensional characteristics of galleries from different trees were remarkably similar, although the numbers of instars varied. The minimum number of instars was six and the maximum twelve. Different numbers of instars were found within the same billets and in galleries originating from the same oviposition drill shaft, with the consequence that the size of adult emergents from a given billet could be uniform or variable, large or small.

The average bulk density of infested wood and insect frass was $0{\cdot}41 \pm 0{\cdot}08$ ($n = 61$) and $0{\cdot}15 \pm 0{\cdot}02$ ($n = 17$) g cm^{-3} respectively, i.e. an apparent utilization of 63%. Highly significant correlations between larval (gallery) and head capsule widths and larval weight, and between size (width) and live and dry weights of larvae, were obtained. These correlations may be summarized as follows:

When x is larval weight in grams and	
y is body width in centimetres:	$\log y = -2{\cdot}37 + 5{\cdot}78x$; $n = 25$; $r = 0{\cdot}96$
y is head capsule width in centimetres:	$\log y = -2{\cdot}36 + 3{\cdot}84x$; $n = 25$; $r = 0{\cdot}99$
When x is head capsule width in centimetres and	
y is fresh weight in grams:	$\log y = -2{\cdot}56 + 7{\cdot}14x$; $n = 22$; $r = 0{\cdot}97$
y is dry weight in grams:	$\log y = -3{\cdot}30 + 8{\cdot}56x$; $n = 22$; $r = 0{\cdot}95$

The diameter of emergence holes was not significantly different to the diameter of the larval gallery in which pupation took place.

On the basis of these relationships, the ratio of the average increment in dry weight during an instar relative to the estimated dry weight of wood displaced was used as an index of utilization, even though the wood was not directly utilized but provided the substrate for the true food, the fungus. The data obtained from dissected galleries from both culture billets (generally small adult emergents) and burnt trees (yielding large adults) were compared (Table 2). The increment in weight per instar relative to the weight of wood displaced showed the same general relationship within each group. However, the number of instars required to achieve a particular size varied.

The average number of instars in billets yielding large and small insects was twelve and ten respectively. However, on the basis of size at a moult, the larvae in the culture billets (small adults) reached their maximum size after ten moults but larvae in field billets (large adults) achieved an equivalent size after only seven moults. Comparison of size and weights of wood displaced revealed three precocious moults preceding instars 3, 8 and 10 in the culture billets, because addition of the successive weights of wood displaced at these moults gave values similar to that displaced by the equivalent instar in the field-collected billets. The size of adult emergents was directly related to the volume of wood displaced (Fig. 6).

Table 2. Gallery width, volume and weight of wood displaced, and size of *S. noctilio* instars within wood producing large and small adults

Egg or larval instar	Head capsule width (cm)	Gallery width (cm)	$10^{-3}\times$ dry wt of stage (g)	Weight increment (g) (A)	$10^{-3}\times$ vol. of wood (cm^3)	$10^{-3}\times$ wt of wood (g) (B)	Ratio A : B
	Wood from field-collected (burnt) trees, yielding large adults; means of 11 galleries						
Egg	–	0·020	0·0700	–	–	–	–
I	0·0139	0·025	0·6653	0·5953	0·06	0·025	23·8120
II	0·0207	0·045	0·7603	0·0950	0·37	0·370	0·2570
III	0·0271	0·068	0·8610	0·1007	1·05	0·431	0·2340
IV	0·0350	0·100	1·0090	0·1480	3·60	1·476	0·1000
V	0·0432	0·137	1·1860	0·1770	9·40	3·854	0·0459
VI	0·0596	0·223	1·6730	0·4510	38·80	15·908	0·0280
VII	0·0769	0·330	2·3070	0·6700	122·10	50·661	0·0130
VIII	0·0834	0·426	2·6180	0·3110	263·20	107·912	0·0028
IX	0·1069	0·538	4·0930	1·4750	670·00	274·700	0·0054
X	0·1208	0·645	5·4700	1·3770	1034·00	423·940	0·0033
XI	0·1213	0·650	5·5340	0·0640	1015·60	416·396	0·0022
XII	0·1361	0·775	7·3960	1·8620	1992·30	816·843	0·0022
	0·1318	0·740	–	–	–	–	–
	Wood from culture billets, yielding small adults; means of 8 galleries						
Egg	–	0·020	0·0700	–	–	–	–
I	–	0·023	0·6564	0·5864	0·02	0·0032	71·510
II	–	0·034	0·7089	0·0525	0·07	0·0287	1·829
III	–	0·046	0·7640	0·0551	0·20	0·0820	0·672
IV	–	0·066	0·8549	0·0909	0·80	0·3280	0·277
V	–	0·096	0·9893	0·1344	2·60	1·0660	0·126
VI	–	0·133	1·1630	0·1737	8·20	3·3620	0·052
VII	–	0·172	1·3610	0·1980	14·70	6·0270	0·033
VIII	–	0·208	1·5530	0·1920	42·20	17·3020	0·011
IX	–	0·247	1·7800	0·2270	46·00	18·8600	0·012
X	–	0·300	2·1170	0·3870	184·50	75·645	0·005
XI	–	0·370	1·8620	–	–	–	–

The direction of larval galleries differed significantly between the upper and lower portions of trees: at 2–4 m, $21\cdot20\pm9\cdot15$ (mean $\pm$ SD) larvae were tunnelling upwards and $10\cdot80\pm5\cdot54$ downwards; at 12–14 m, $10\cdot40\pm3\cdot34$ were tunnelling upwards and $15\cdot60\pm4\cdot22$ downwards ($P<0\cdot05$ in both cases). This indicates that patterns of drying in different regions of trees influences larval behaviour.

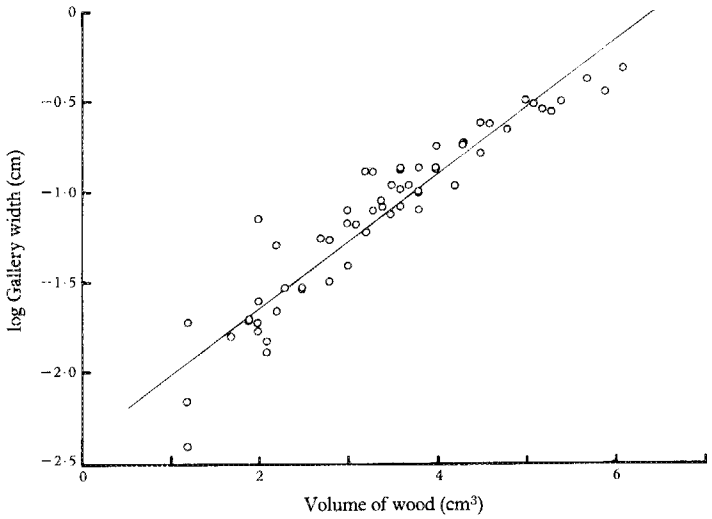

Fig. 6. Relationship between the volume of wood displaced and the size of the larva. Regression equation: $\log y = -2{\cdot}38 + 3{\cdot}69x$; $n = 63$, $r = 0{\cdot}94$.

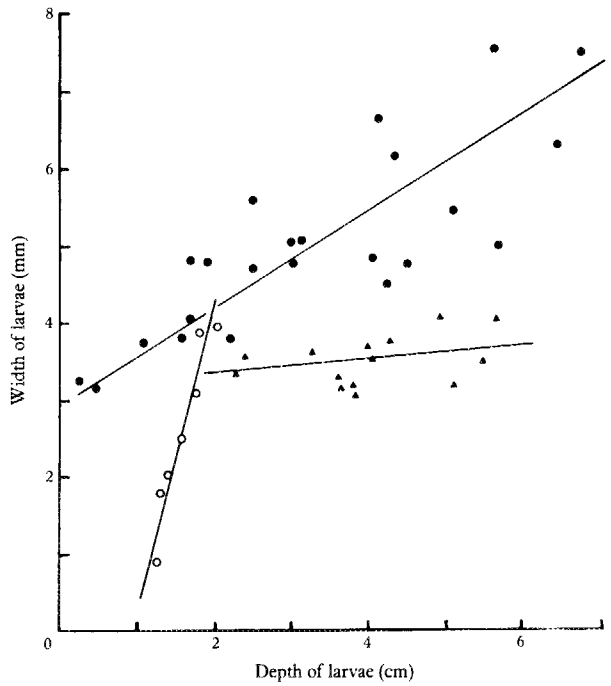

Fig. 7. Relationship between the diameter and depth of larval space in billets with a moisture content of 130% (○), 75% (▲) and 46% (●).

There was no difference in the shapes or characteristics of galleries between those made by male and female larvae, and though larger larvae tended to lie deeper than smaller larvae this was not always so. In some trees with high internal moisture contents small and large larvae were confined to the outer perimeter just beneath the bark, and in many dry trees small larvae were found deep in the wood (Fig. 7).

Feeding Behaviour of Parasitized S. noctilio *Larvae*

(i) *Methods*

Sirex-infested billets containing either the cynipid parasitoid *Ibalia leucospoides* Hochenw, or the ichneumonid parasitoids *Rhyssa persuasoria* and *Megarhyssa nortoni nortoni* Cresson, were dissected and the location of parasitoids or parasitoid-infested larvae compared to that of unparasitized larvae.

(ii) *Results*

Parasitism by *I. leucospoides* affected the development and growth of *Sirex* larvae. Such larvae had completed fewer instars and were located at, usually, shallower depths than unparasitized larvae. However, this relationship was modified by the moisture content in the wood. Eight initially wet, infested billets, 2 m long and of 20 cm maximum diameter, were held vertically in laboratory cubicles. After 7 months, the interface between wet and dry wood was at a mean depth of $7{\cdot}24 \pm 1{\cdot}03$ cm from the outer surface at the top of the billets, and at $4{\cdot}83 \pm 0{\cdot}51$ cm at their bases. The mean depth of unparasitized and parasitized *Sirex* larvae was $5{\cdot}08 \pm 0{\cdot}76$ and $2{\cdot}80 \pm 0{\cdot}88$ cm, respectively, at the top, and $2{\cdot}29 \pm 0{\cdot}46$ and $1{\cdot}78 \pm 0{\cdot}19$ cm at the base. Thus, gravitational settling of water had restricted fungal growth, and hence larval infestation, at the base of each billet to the outer perimeter. The unparasitized larvae were also larger at the top than at the base.

Parasitism by the ichneumonids resulted in the rapid immobilization and consumption of the host larvae and hence had no effect on gallery size.

Evidence of parasitism by both groups of parasitoids was, in the absence of live stages, given by a reduction in the diameter of the emergence gallery and hole compared to that expected.

Chemical Properties of Sirex*-infested* P. radiata

Methods

The carbohydrate and nitrogen status of *Sirex*-infested and non-infested *P. radiata* was compared by means of the Priestley (1962) and Kjeldahl (in Vogel 1948) techniques respectively. The infested wood consisted of: (1) culture billets with small larvae; (2) an infested tree with small to medium-sized larvae; (3) a tree from which adults had emerged 4 months previously. A minimum of 12 wood samples were removed from both infested and non-infested materials, for assessment of moisture contents. Moisture content was also determined of frass at 1-cm intervals along the lengths of six galleries in the infested wood.

(ii) *Results*

Measurements of carbohydrate and nitrogen levels indicated progressive reductions in all components with time from that of the original infestation (Table 3). The nitrogen content in larval frass in the trees containing actively feeding larvae ranged from 0·12 to 0·20%, in comparison to 0·02–0·04% in wood infested with

the fungus ($n=6$). In the wood adjacent to the larval gallery the nitrogen content ranged from 0·06 to 0·09%; this wood had a 'soaked' appearance but its moisture content was similar to that in the adjacent clear wood. The soluble carbohydrate level in frass was 1·45–1·65%, compared to 1·74–1·86% in fungus-infested wood. The moisture content also differed markedly between frass and the surrounding wood, and the moisture content of frass declined along the length of the gallery. These differences were more marked in those galleries containing actively feeding larvae. The average moisture content of frass 1 cm behind actively feeding, mature larvae was 100±14% ODW, compared to a general wood moisture content of 70±20% ODW. In contrast, in billets of initially low moisture content, in which larvae were feeding more slowly, the average moisture contents in frass and wood were 28·2±2·4% and 20·0±11·6% respectively.

Table 3. Moisture content, and carbohydrate and nitrogen status, of wood in relation to stage of *Sirex* infestation

Values for carbohydrate are single estimates from five pooled samples per treatment; those for nitrogen are from four samples per treatment

Quantity	Control billets fresh, uninfested	Culture billets, small larvae	Infested tree, small–medium larvae	Tree with emergence
Moisture content (percentage of dry wt)	179	147	113	47
Carbohydrates (percentage of residual dry wt)				
Soluble sugars	8·09	4·43	2·70	1·79
Starch	4·09	2·51	3·18	2·76
Hemicellulose	20·02	19·50	17·51	20·02
Total per 100 g	32·10	26·44	23·39	24·07
Nitrogen (percentage)	0·057	0·053	0·043	0·038

Growth of the Symbiotic Fungus Amylostereum areolatum *Boidin*

(i) *Methods*

Pure cultures of *A. areolatum* were established on potato dextrose agar (PDA). These cultures were subcultured by transferring 0·3-cm plugs to new plates and incubating at 22°C. When the floor of the new plate was colonized the perimeter was marked on the undersurface and five plates incubated at 5°C intervals between 5 and 30°C. Radial growth was recorded every 7 days, and the growth rates at the different temperatures assessed from the average incremental values.

(ii) *Results*

A. areolatum failed to grow at the extreme low and high temperatures. The optimal temperature for growth was 25°C and the threshold value was 6·2°C. The thermal constant for growth of 5 cm radially was 661 day-degrees.

Discussion

The rate of development of *S. noctilio* eggs was dependent on temperature within the range 10–25°C. The estimated threshold temperature was 6·2°C, which was similar to that assessed for the symbiotic fungus *A. areolatum*. Such a minimum temperature could occur during the winter months in Tasmania, in the bases of large trees under canopy; dormant eggs have been found in such trees 10 months

after oviposition (K. L. Taylor, personal communication). However, such temperatures would not prevail through the year, so other processes must operate to inhibit egg development. The coincidence of the threshold temperature for eggs and fungus, and the facts that fungal growth always precedes egg development and that the fungus is limited by high moisture contents in wood (Coutts 1965), suggest that volatile by-products of the fungus may initiate embryogenesis. The respiration of wood blocks containing eggs was enhanced, but declined at the time of eclosion; exposure of eggs to carbon dioxide accelerated development. This promotion of development was more pronounced in those eggs which had been deposited 'free' of wood and hence of initial fungal effects. *A. areolatum* also produces ethanol (unpublished information). However, the retardation in development of eggs briefly immersed in ethanol may also reflect changes in the wet and anoxic basis of trees.

Complete development of *S. noctilio* was also dependent on temperature over the range 12·5–33·5°C. In Tasmania the majority of *S. noctilio* emerge 12 months after oviposition, and the remainder emerge in the second season, i.e. 24 months after oviposition (Taylor 1978); in Europe *S. noctilio* may emerge over four consecutive seasons (Spradbery and Kirk 1978). In contrast, the minimum time observed for adult emergence was 8–10 weeks following incubation of billets at 33·5°C.

Embryogenesis could not be followed in detail, as eggs were directly observed and the embryo was obscured within the yolk for much of the developmental period. However, the sequence outlined does agree with that reported by Anderson (1972) for the Hymenoptera in general and there was no evidence of blastokinesis.

Within the log, the larvae turn within the drill shaft after eclosion to consume egg remnants, and then graze on mycelial filaments which by that time have penetrated the shaft. The fungus arises from arthrospores deposited by the *S. noctilio* female in an egg-free, adjacent shaft (Madden 1974). Two moults may occur within the drill shaft but in most instances the first moult just preceded the larvae entering the tracheids, therefore the first-instar larvae fed on only egg and fungal material, not wood. This concentrated form of food resulted in a high conversion ratio for first-instar larvae, which is, however, only apparent, for it is based on the assumption that nutrition was derived directly from the wood displaced. The higher apparent ratio in culture billets than in field trees resulted from the fact that within the shafts of culture billets movement of larvae was restricted, which resulted in a lower volume or weight denominator in the computation.

The conversion ratio progressively declined throughout the instars until larval weight did not increase. Contrary to the findings of Boros (1968) and Morgan (1968), fragments of wood were found in the midgut of active larvae. These components were made visible by staining with phloroglucinol for lignin and lactophenol–cotton blue for mycelium. All larvae ceased feeding before pupation and retained no particulate matter within the midgut (Madden 1975). This resulted from the limitation of fungal growth through progressive reductions in moisture, carbohydrate and nitrogen levels in the wood biomass. The accumulation of nitrogen around the gallery and within the frass, together with enhanced moisture contents of frass, indicated the mobilization and transport of these materials by

the fungus from the general infested wood to the larval space. The grazing activity of the larvae presumably maintained this transport by stimulating fungal growth.

Microorganisms occur within the gut of actively feeding larvae and play a probable role in nutrition (Madden 1975). However, the presence of *Azotobacter* sp. does not appear to be responsible for the high nitrogen levels observed, because the *Rf* values of ninhydrin-positive compounds within the frass were comparable to those of the fungus (unpublished information).

The ultimate size of *S. noctilio* adults was determined by conditions within the tree, which influenced fungal growth. The drying patterns in the tops and bases of the same trees affected the proportions of larvae which tunnelled either up or down these trees. For the same reasons, two or more larvae which originated from eggs deposited in the same drill shaft could emerge as adults at the same time but be of very different sizes.

Therefore the length and shape of the larval gallery was related more to the performance and degree of invasion of the fungus than to the larvae *per se*. Moulting may follow the attainment of a particular minimum size and, with the exception of the first and sometimes the second instar, was attained without obligatory turning, as claimed by Rafes (1960). However, moulting was accompanied by a change in direction if the larvae encountered resin pockets, dense branch nodes or other larval galleries.

The tunnel shapes described by Rafes (1960, 1961) undoubtedly reflect conditions of a more extreme nature than those encountered in Tasmania. The results reported have indicated that larval behaviour and development were dictated by conditions which influenced fungal growth rather than by innate behaviour patterns of the larvae themselves. A similar interpretation may well apply to other siricid species and wood-inhabiting insects with a symbiotic fungus relationship.

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Haematolo
Serum Bio
Trichosurus
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P. J. A. Pres
[A]Department of
Werribee, Vic,
[B]Present addres
Road, Westmea
[C]Present addres
Santiago, Chile.

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