ECOLOGY OF *Hylecoetus dermestoides* LINNAEUS

(COLEOPTERA:LYMEXYLIDAE)

IN NORTH EAST SCOTLAND

by

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TO MY PARENTS,

BROTHERS AND SISTERS

ABSTRACT

During 1983–1986 studies on the life cycle, emergence, flight and weather, host selection and ambrosia association of a population of *Hylecoetus dermestoides* Linnaeus (Coleoptera:Lymexylidae) breeding in stumps and standing dead trees of European larch (*Larix decidua* Miller) were carried out at Dunkeld, Perthshire, Tayside Region (in both Atholl and Craigvinean forests), and in the Department of Forestry and Natural Resources, University of Edinburgh.

The life cycle was investigated by following the development of *Hylecoetus* under field conditions and by rearing *Hylecoetus* under controlled conditions in naturally infested pieces of wood, and on agar at 20°C. Larval instars were determined: (1) by measurements of head capsule widths and the application of Dyar's rule; and (2) by counting moulted head capsules on agar. Five instars for female and 5 instars for male *Hylecoetus* were determined. The pupal period is about 7 days at 20°C. The life cycle is completed in 1–2 years. Completion of the life cycle in 9 months was achieved at 20°C. in the laboratory.

Emergence of *Hylecoetus* was studied by using caged naturally infested pieces of wood in the field. Emerging *Hylecoetus* were collected on a daily basis. Flight was studied by counting the numbers of *Hylecoetus* responding to standing dead trees, stumps, logs and semiochemicals. Window barrier, cross-vane and multiple funnel traps were used to catch responding *Hylecoetus*. *Hylecoetus* were collected from trap sites on an hourly and daily basis respectively. Emergence and flight coincided and occurred from the end of April to the end of June, and were at their maximum from mid-May to mid-June. Emergence and flight were correlated with favourable weather. The

temperature threshold for flight is about 15°C. Sunshine hours (or light intensity) is positively correlated with flight. The number of hours during which the temperature exceeded the threshold for flight and temperature range above 15°C. were also positive correlates of flight. Relative humidity, wind speed and rainfall were negative correlates of flight. *Hylecoetus* are day flyers and have unimodal diurnal flight curves. Diurnal flight occurs from 10.00-20.00 HRS with the main flight being at 13.00-15.00 HRS and peaks at 15.00 HRS. Frequently, in the morning, temperatures below the threshold for flight, high humidities and high wind speeds prevented flight, whereas in the evening high humidities, high wind speeds and low light intensities prevented flight.

Emerging *Hylecoetus* have approximately a 1:1 sex ratio. 175 all-black and 21 yellowish brown males were examined (ratio=8.3:1). Pairing and oviposition were recorded in the field throughout the 1984 season; 23% and 77% of female *Hylecoetus* laid eggs on the day of, and the day after emergence respectively. Eggs were laid in 1-3 batches, and a total of 7-61 (mean=29) eggs per female. Ectosymbiotic fungus-free females in culture laid eggs 1-2 days after emergence, but had fewer (mean=18) eggs than females from natural populations.

There was variability in the attractiveness of logs, stumps and standing dead trees to *Hylecoetus*. Ethanol, with or without camphene was attractive to *Hylecoetus*. Mate location or courtship seems to employ a secondary attraction process involving tactile, visual and chemical cues.

Ceratocystis piceae (Munch) Bakshi was isolated as the sole ambrosia fungus for the Scottish race of *Hylecoetus*, being found in larval tunnels, larval guts and adult *Hylecoetus*. Common laboratory agar media were used: potato dextrose agar (PDA); corn meal agar (CMA); malt extract agar plus yeast extract (YEME); malt extract agar (MA) and Czapek dox agar (modified) (CzDA).

Hylecoetus ingests a wood-*C. piceae* complex which has more amino acids than fresh bark and sapwood respectively, and is also a source of sugars and other factors required for growth and reproduction. More amino acids were found in larch bark than sapwood. *C. piceae* grows more rapidly on bark than sapwood; and more rapidly on sapwood than heartwood meals respectively. Based on rates of linear extension on agar, arginine, leucine and cystine; sucrose, glucose and fructose seemed to be important organic nitrogen and carbon sources respectively for growth of *C. piceae* in culture. Temperature between 15° and 25°C. and acid medium are favourable for growth of *C. piceae* in culture.

The study also includes (1) literature review pertaining to *Hylecoetus* biology which includes life cycle, emergence and flight, host selection, ambrosia association and importance of *Hylecoetus* as a forest pest (2) determination of amino acids and sugars in larch bark, sapwood and *Hylecoetus* bore-meal using thin layer chromatography (TLC) and High Voltage Electrophoresis (H.V.E.P.) for qualitative determinations and ion-exchange chromatography for quantitative determinations.

DECLARATION

This thesis has been composed by myself and it has not been submitted in any previous application for a degree. The work reported herein was executed by myself, unless otherwise stated.

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INTRODUCTION

Hylecoetus dermestoides Linnaeus (Coleoptera:Lymexylidae) is a wood-boring species. Lymexylids have a worldwide distribution and have been studied for a long time due to their boring activity and associated fungal infections. *H. dermestoides* has been recorded on the Continent of Europe including Britain, in Russia, Australia, United States and Japan. *Hylecoetus* attacks standing dead trees, green logs, stumps of felled timber and branches of a wide range of conifer and broad-leaved tree species. *Hylecoetus* can cause considerable damage to timber and make it unfit for many purposes. *Hylecoetus* attacks are characterized by tunnels with a wide range of diameters made in the sapwood by larvae; small heaps of white-coarse bore-meal on the bark (Fig:1), and when bark is peeled or part of the sapwood is removed larvae, pupae or adult *Hylecoetus* may be found (Fig:2). The *Hylecoetus* larva has a sclerotized spine on the ninth abdominal tergite, a feature which makes it impossible for the larva to be confused with that of any other insect (Fig:3). Larval tunnels are lined with ambrosia fungi upon which the larvae feed and derive their nourishment. Ambrosia fungi are carried from one generation to another by adult *Hylecoetus*. Fungal spores are transmitted in specialized structures (mycangia) in female *Hylecoetus* and are introduced into galleries by larvae during the burrowing process by first stage larvae. Adult *Hylecoetus* select hosts which provide a suitable environment for larval and fungal growth. In the wood the ambrosia fungi live on mainly the xylem sap and synthesize important vitamins and supply these together with amino acids and other growth requirements to their insect associates. Quantitative data on the roles of *Hylecoetus* and ambrosia fungi in this symbiosis are not available.

The life cycle of *Hylecoetus* has not been well studied. It is thought that

Fig: 1. *Hylecoetus dermestoides*. Larval tunnels in a conifer stump.

(a)

(b)

Fig: 2. (a) Split stump of European larch (*Larix decidua* Miller) showing specimens of *Hylecoetus*; (b) first instar larva of *Hylecoetus*.

(a)

(b)

Fig: 3. (a) A characteristically middle instar larva of *Hylecoetus*, and (b) last instar larva and pupa on agar (note the absence of fungal growth).

the life cycle is completed in 1-2 years. Number and duration of larval instars are not known. *Hylecoetus* flight mostly occurs from the end of April to the end of June. Female *Hylecoetus* lay eggs in heaps, in crevices in the bark (Fig:4).

Control of *Hylecoetus* has been little studied. Control measures tried, so far, have included management practices such as debarking logs, hot-logging, and use of chlorinated hydrocarbons, e.g., gamma BHC or dieldrin. More recently work on the control of *Hylecoetus* using semiochemicals i.e. insect pheromones and host tree-produced kairomones was re-initiated in Europe. Some of the natural enemies of *Hylecoetus* have been identified, but their role and efficacy in the biological control of *Hylecoetus* have not been studied.

Knowledge about the biology of a forest insect pest is fundamental to the planning of control strategies. Information about the habits of the insect pest and other biological factors associated with the pest, such as its natural enemies should be sought so that an insight into the effects of control measures on both the target and non-target species can be obtained. Biological control of pests may be incorporated in integrated pest control programmes. Timing of control measures should take advantage of natural enemies of the target species. That is, if we know the time of the year the pest species is attacked by parasites and predators, then we may be able to direct control measures against the pest species when the natural enemies are not available, and thus the control would be more effective. In determining the length of time available for application of such favourable control before the appearance of any particular parasite for which protection is desired, it is necessary to ascertain which stage of development of the host is preferred by the parasite, and also to know exactly when this stage of the host occurs. This calls for a thorough knowledge of the different stages of development of the pest species and its natural enemies.

Fig:4. Female *Hylecoetus* ovipositing in a crevice on a larch standing dead tree.

Biological studies of adult wood-boring Coleoptera are generally concerned with the determination of the time of the year adults occur and their habits in relation to their environment. Since most wood-destroying Coleoptera spend most of their development in wood and usually have to fly to new hosts to breed, this entails studying the emergence and flight patterns. Knowledge about emergence and flight patterns of wood-destroying species is important for optimal scheduling of logging activities and for log protection procedures. Flight activity is usually divided into two categories: trivial flights and migration. Trivial flights are local movements concerned with the finding of food, a mate, or suitable oviposition site or escaping from a potential enemy. Migration means those flights in which vegetative activities are suppressed so that flight behaviour dominates, frequently carrying the insect beyond the confines of its habitat, and it is regarded as a dispersal mechanism. Quantitative data on the emergence and flight activity of the Scottish race of *Hylecoetus* are lacking.

Ambrosia beetles use complex systems or processes in which both insect-originating pheromones and host tree-produced kairomones are used to locate mates and suitable ovopisition sites. Knowledge of natural insect attractants has led to the production of synthetic chemical attractants which have a range of applications: in survey and detection programmes; delimitation of an infestation; evaluation of the density of insect populations, and predicting density in the next generation; and to reduce populations by trapping and disrupting mating. Other uses include chemical baits which lure insects to traps, where the insects become contaminated with a virus or other pathogen. After mating the other insects are in turn contaminated with the virus which is spread to the eggs. At present there is little information on host-derived attractants and pheromones in *Hylecoetus*. However, very recently, ethanol and a female *Hylecoetus*-derived cyclic enolether

2,5-dimethyl-2-isopropanol-2,3-dihydrofuran have been identified as attractants of *Hylecoetus*. The work on pheromones of *Hylecoetus* and its other natural attractants may lead to isolation, identification, synthesis and testing of attractants for *Hylecoetus* which could be used in trap-out strategies to contain *Hylecoetus* numbers. However, undertaking such extensive work can be justified only by the presence of economically important *Hylecoetus* damage.

Although Continental records indicate that *Hylecoetus* has an ambrosia association, the nature of the nutritional symbiosis between the microorganisms and *Hylecoetus* remains obscure. Study of the nutrition of the Scottish *Hylecoetus* has lagged behind similar studies of other important species. All that is known is that the *Hylecoetus* larva bores in wood, and whether or not the larva actually uses wood directly for its nourishment, or whether or not the larva is intimately associated with symbiotic microorganisms remains entirely unknown.

Available information on *Hylecoetus* is based on studies carried out mostly on the Continent of Europe; thus the Scottish race of *Hylecoetus* remains virtually unexplored. Given this background it became pertinent to investigate the following problems: life cycle; emergence and flight patterns and weather; host recognition and colonisation; and the ambrosial association in *Hylecoetus*.

CHAPTER 2

CHAPTER 2

LITERATURE REVIEW

2.1. *Hylecoetus dermestoides* Linnaeus: A review of the biology of the insect.

2.1.1. Systematics

H. dermestoides is a member of the Coleoptera, belonging to the suborder Polyphaga (the omnivorous beetles). Within the suborder, *H. dermestoides* has been described as belonging to the Series Cucujiformia and the superfamily Lymexylonidea (Crowson, 1981). The latter is subdivided into two families, the Lymexylidae (synonym: Lymexylonidae) and the Stylopidae. Freude, Harde and Lohse (1979) placed various taxa of 23 families including Lycidae, Lampridae, Cantharidae, Malachiidae, Melyridae, Cleridae, Lymexylonidae, Elateridae, Eucnemidae, Buprestidae and Dermestidae in the superfamily Diversicornia. Earlier literature (Linssen, 1959) shows that the family Lymexylidae belonged to the superfamily Serricornia and later Diversicornia and the series Malacodermata. Tillyard (1926) described the family as belonging to the Superfamily Lampyroidea (Malacodermata).

Members of the family Lymexylidae are wood-borers (or wood-destroyers). These are long cylindrical beetles with soft integuments. The maxillary palpi are flabellate in the male. The front and middle coxae exerted, longitudinal in position; tarsi slender, five-jointed; antennae short, serrate, but rather broad. The larva has an enlarged prothorax, well-developed legs and the ninth abdominal tergite though often greatly modified does not bear paired processes. There are said to be six Cryptonephric Malpighian tubules (Crowson, 1981). Although a small family with 37 (Britton, 1973) known species it has representatives in almost all parts of the world, which are remarkable on account of their destructive habit of drilling cylindrical holes in hardwood, and

also because of associated fungus infections.

There are four described genera in the family Lymexylidae: *Hylecoetus, Melittomma, Lymexylon and Atractocerus*. In *Atractocerus* the antennae are very short and serrate and the elytra being rudimentary do not exceed beyond the thorax. The exposed hind wings are broad and fold fan-like and extend to about half the length of the long, narrow and, flattened, dark brown abdomen. The metathoracic spiracle in *Atractocerus* is unusual in being completely exposed and tibial spurs are absent. Adults of a species of *Atractocerus* in West Africa are reported to fly in swarms at dusk and are sometimes attracted to light. *Melittomma insulare* Fairmaire has been recorded as a major pest of coconut in the Seychelles (Brown, 1954). *Lymexylon* and *Hylecoetus* contain species in which the elytra completely cover the body. According to Linssen (1959) in Britain the family Lymexylidae is represented by two genera and two species. The two species are *H. dermestoides* and *Lymexylon navale* L. These species are elongate in shape and closely covered with a short pubescence, and the elytra are finely punctured. A peculiar characteristic of these insects is the maxillary senseorgan of the male, (figured in Linssen, 1959 and Schwenke, 1974). The tarsi are also remarkable, being long and thread-like. The difference between the two species is most clearly marked in the antennal structure, which is serrate in *H. dermestoides* and filiform in *L. navale*; further more the thorax is transverse in the former species but quadrate in the latter. Larvae are very distinctive in appearance and are figured in Schwenke (1974).

There are six described species of *Hylecoetus*. The species are distributed as follows *H. lugubris* Say New York (USA), *H. pervagus* Oll., Australia and both *H. dermestoides* and *H. flabellicornis* Schnd. are found in Europe. *H. cossis* Lewis, *H. matsushitai* sp. n. and *H. flabbellicornis* are found in Japan (Kôno, 1938a,b).

The generic name *Hylecoetus* was originally used in 1806 by Latreille and, the species name *dermestoides* by Linnaeus in 1761 (Joy, 1932; Kloet and Hincks, 1977). In Britain the larva of *H. dermestoides* is commonly known as the large timber worm (Seymour, 1979).

2.1.2. Distribution and host plants of *H. dermestoides*

2.1.2.1. West Germany

Conifers

H. dermestoides and *Xyloterus lineatus* (Olivier) were recorded as secondary pests of silver firs in the Frankewald by Scheidter (1919). Bletchly and White (1962) noted that *H. dermestoides* was a secondary pest of *Pinus*.

Broadleaves

Reichling (1920) reported that *H. dermestoides* and *X. lineatus* were secondary pests of trees which had been primarily attacked by the Beech Scale, *Cryptococcus fagi* Baerensprung in Westphalia. After insect attack the trees were infested by a fungus, *Nectria ditissima*. Zimmermann (1973) recorded *Hylecoetus* attacking beeches *Fagus sylvatica* L. in the Hanover District.

The occurrence of *H. dermestoides* in Germany has also been reported by Freude *et al.* (1979), Francke, Mackenroth and Schrober (1984).

2.1.2.2. Switzerland

The Lymexylidae are represented by two species, *H. dermestoides* and *L. navale*. Larvae of *Hylecoetus* were reported boring in the sapwood of beech stumps, and they were occasionally found in standing trees; *L. navale* preferred oak (Schneider-Orelli, 1920).

2.1.2.3. Austria

Conifers

H. dermestoides was reported attacking Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) in Vienna by Schwarz (1933). Kurir (1972) and Egger (1974) reported that *H. dermestoides* attacks the following tree species destroyed by snow avalanches in areas with altitudes from 1000 to 1500 m.: *Picea abies* (L.) Karst., *P. orientalis* (L.) Link., *Abies alba* Mill., *Pseudotsuga menziesii, Larix decidua, Pinus sylvestris* L. and *P. nigra* var. *palassiana* (D. Don) Holmboe.

Broadleaves

Among the species attacked are: *Juglans regia* L., *Carpinus betulus* L., *Betula verrucosa* Ehrh., *B. pubescens* Ehrh., *B. tortuosa, Alnus* sp. *Fagus sylvatica, F. orientalis* Lipsky, *Quercus* sp., *Q. robur* L., *Q. petraea* (Matt.) Lieblein., *Q. pubescens* Willd. (Synon. *Q. lanuginosa* Thuill.), *Q. cerris* L., *Prunus* sp., *P. avium, Acer* sp. and *Fraxinus excelsior* L. (Kurir, 1972; Egger, 1974).

2.1.2.4. Finland

Conifers

Another species, *Hylecoetus flabellicornis* attacks spruce stumps (Löyttyniemi, 1967; Löyttyniemi and Uusvaara, 1977).

Broadleaves

H. dermestoides attacks birch (*Betula* sp.) only (Rummukainen, 1947; Saalas, 1949).

2.1.2.5. Denmark

Broadleaves

Thomsen, Buchwald, and Hauberg (1949) and Thomsen (1950) report *H. dermestoides* as a secondary forest pest attacking standing diseased beech in Denmark. The insect species also attacked walnut. Beech trees (*Fagus silvatica*) had primarily been attacked by the Beech Scale or Felted Beech Coccus *Cryptococcus fagi*, and secondarily by fungal infection caused by *Nectria galligena* Bres. The adult *H. dermestoides* attacked the beech trees, and the hatched larvae burrowed long, narrow galleries in the wood, penetrating sometimes to the centre. Heavy attacks, mostly in the lower part of the bole were noted. *H. dermestoides* attacked the beech trees together with another wood-boring species *Xyloterus domesticus* (L.)(Scolytidae). Both species damaged the timber severely making it unfit for many purposes, and facilitated the invasion of sapwood-destroying fungi, the most common of these species being *Stereum hirsutum* (Willd) Pers, and *Polyporus adustus* (Willd)Fr. At a later stage, when the sapwood was already destroyed, the fruit bodies of heartwood-destroying fungi appeared, the most important being *Fomes fomentalis* (L.) Gill and *F. ungulatus* (Schaeff.) Sacc. Other species observed were *Polyporus radiatus* (Sow.) Fr. and *Armillaria mucida* (Schrad.) Quél.

2.1.2.6. Sweden

Broadleaves

H. dermestoides and *Agrilus viridis* L. (Buprestidae) were reported as secondary pests of birch (*Betula tortuosa* auct.), the dying trees having been severely defoliated in 1954-56 by an out break of the Geometrid, *Oporinia autumnata* (Bkh.) (Palm, 1960).

Conifers

H. dermestoides has been found in timber of *Pinus* spp and spruce (Bletchly and White, 1962).

2.1.2.7. Other areas on the Continent

H. dermestoides occurs in Norway (Lyngnes, 1958), Holland, Spain (Bletchly and White, 1962) and Poland (Capeck, 1982).

2.1.2.8. Siberia

H. dermestoides has been recorded from a number of hardwoods and softwoods (Bletchly, 1967).

2.1.2.9. Australia

The family Lymexylidae is represented by nine species belonging to four genera *Melittomma*, *Lymexylon*, *Hylecoetus* and *Atractocerus*. The species *Hylecoetus pervagus* is well-known there (Tillyard, 1926). However, Britton (1973) believes that the Australian species are not congeneric with *Hylecoetus* of the Northern hemisphere and should be transferred to another genus.

2.1.2.10. United States

Broadleaves

Snyder (1927) reported *Melittomma sericeum* Harr. attacking chestnut and red oak, while other ambrosia beetles, *Corthylus columbianus* Hopkins, *Platypus compositus* Say, *Xyleborus* spp. and *Monathrum* spp. attacked chiefly oaks and poplars, *Eupsalis minuta* Drury in oak, and *Strongy* spp. in various hardwoods. *Xyloterus* sp. occurred in felled trees. *Hylecoetus lugubris* oviposited in dying trees and green saw logs with bark on which were lying in the forest from April to July.

Conifers

H. lugubris has been reported attacking *Pinus resinosa* Ait. in Ithaca, New York (Batra and Francke-Grosmann, 1961; Batra, 1963a, 1967).

2.1.2.11. Japan

Conifers

Hylecoetus cossis Lewis, *Monachamus sartor* var. *rosenmuller* Ceder, and various Scolytids and weevils were reported infesting weakened trees and sometimes the attacked trees (*Abies sachalinensis* Mast. and *Picea jezoensis* (Siebold and Zuccariai) Carrière) died (Kôno, 1938a). *Hylecoetus matsushitai* was reported attacking *Abies sachalinensis* and *Picea jezoensis* in Sakhalin and Hokkaido, and *H. (Hylecerus) flabbellicornis* attacking *P. jezoensis* in Sakhalin, (Kôno, 1938b).

2.1.2.12. Britain

A long series of records dating from 1889 indicate that *H. dermestoides* is indigenous to Britain and that *H. dermestoides* is generally restricted to conifers in Scotland and to broadleaves in England (Fowler, 1889; Fergusson, 1920; Munro, 1926; Joy, 1932; O'Mahoney, 1934; van Emden, 1943; Sandars, 1946; Bakshi, 1950; Linssen, 1959; Bletchly and White, 1962; Bletchly, 1967; Luff, 1970; Kloet and Hincks, 1977; Seymour, 1979; Thorpe, 1982; Bevan, 1987).

Scotland

1. *Broadleaves* : *H. dermestoides* was reported by Fergusson (1920) at Balquidder and Dunkeld. Larvae were found in logs of *Prunus avium* L., *Betula* spp. and *Fagus sylvatica*.

2. *Conifers* : At Balquidder and Dunkeld, *H. dermestoides* larvae were found in stumps of *Larix decidua*, *Picea* spp., *Pinus sylvestris*, *Castanea sativa* Mill. Bakshi (1950) recorded *H. dermestoides* occurring with other wood-destroying species e.g. *Tomicus minor* Hartig and *Pityogenes chalcographus* L. He reported the insect attacking dying trees of Japanese larch [*Larix leptolepis* [Sieb. et. Zucc.]

Murr. = *kaempferi* Sarg. Carr.]. Attacks were confined to the lower half of the trees, being heaviest near the base and gradualllv diminishing towards the top. *H. dermestoides* was associated with *Xyloterus lineatus*, *Hylurgops palliatus* and *Dryocoetes autographus*. Bletchly and White (1962) surveyed damage by *X. lineatus* and *H. dermestoides* in windblown timber, logs stacked at the roadside and logs stacked in sawmills in Argyllshire (Map 1). Both insects attacked *Abies procera*, *Larix kaempferi*, *Picea abies*, *P. sitchensis (Bong.) Carr.*, *Pinus sylvestris* and *Pseudotsuga menziesii*. In addition, *H. dermestoides* attacked *Abies grandis* Lindl.

2.1.3. Economic importance of insect and damage in forestry

In Scotland logs and stumps from winter fellings become more susceptible to attack in spring and summer (Bletchly and White, 1962). Attack occurs in host material with low moisture contents (Thorpe, 1982). The latter worker found that *Hylecoetus* attacks correlated well with low moisture contents of larch stumps. These findings agreed with those of Bletchly and White (1962). Egger (1974) also found that ovipisition of *Hylecoetus* on beech stumps and stems did not usually take place until one year after felling. In Norway, Lyngnes (1958) had earlier observed that female *H. dermestoides* were not attracted to logs less than three months old, older logs which had been cut about 15 months before being preferred.

Adult *Hylecoetus* do not cause any damage to the attacked hosts but lay eggs in crevices on the bark surface. The hatched larvae tunnel into the bark and sapwood. Larvae seldom tunnel into the heartwood. The entry holes, unlike those made by platypodids and scolytids are initially small but are enlarged at a later stage by the larvae.

Timber infested with *H. dermestoides* can be easily identified by the presence of small heaps of white coarse bore-meal on the bark. Attack is difficult to detect prior to production of the bore-dust. Bore-holes in the wood are variable in size and the wood lacks the discoloration of fungal attack associated with bore-holes of, for example, the ambrosia species, *Xyloterus*

lineatus. *H. dermestoides*'s tunnels do not follow a definite pattern and there are no branch galleries such as are found in the case of *X. lineatus* etc. However, each *Hylecoetus* larva enters the bark and continues boring into the wood and remains in the same gallery until pupation takes place. Depending on the time of the year, when the bark of an infested tree stump or standing dead tree is peeled, larvae, either with their heads still facing the back of the tunnels or facing the entrance, pupae or adult *Hylecoetus*, may be found. During the emergence and flight period enlarged exit holes in the bark may be seen. That the damage is caused by *H. dermestoides* is confirmed by inspection of larvae which have a characteristically long terminal spine with a bifurcated tip. The tip is usually seen expelling frass (Fergusson, 1920).

H. dermestoides has been shown to be a true ambrosia beetle as regards its nutrition (Eckstein, 1929b; Werner, 1929; Thomsen *et al* ., 1949; Thomsen, 1950; Batra and Francke-Grosmann, 1961; Batra, 1963a; Francke-Grosmann, 1963; Batra, 1967; Graham, 1967; Kurir, 1972; Egger, 1974). *Hylecoetus* cultivates a fungus inside its tunnels upon which the larvae feed. Although larval tunnels of typical ambrosia beetles such as *Xyloterus lineatus* are darkly stained by fungal growth, *H. dermestoides* tunnels are less markedly stained. Larval tunnels contain loosely and in some cases, tightly packed bore dust (see Section 2.2).

2.1.3.1. Economic importance of damage

Members of the family Lymexylidae have for a long time been regarded as having no economic significance (Stebbing, 1914; Anderson, 1960; Bletchly and White, 1962; Roberts, 1969; Baker, 1972; Löyttyniemi and Uusvaara, 1977). In Finland, both *Hylecoetus flabellicornis* and *H. dermestoides* attack stumps and lower portions of dying trees. However, since no previous reports have been made about their damage on green timber the two species are regarded as unimportant as pests (Löyttymemi and Uusvaata, 1977). Fergusson, (1920),

reported that from an economic standpoint larvae working in stumps do no harm. However, attacks in logs would degrade the wood. Bletchly and White, (1962), pointed out that although *H. dermestoides* is also wide spread like *X.lineatus* the damage is less apparent due to the absence of associated staining. The lack of staining probably accounts for the greater attention paid to *Xyloterus* attack in Scotland. In Central Europe, *H. dermestoides* is regarded as a harmful pest of green, hard and softwood timber (Schwenke, 1974). Thomsen *et al* . (1949), Thomsen (1950), report *H. dermestoides* attacking beech and degrading the wood, making it unsuitable for sawing or conversion. This activity facilitates the invasion of sapwood and heartwood-destroying fungi in the attacked trees.

2.1.4. Life history

There are numerous Continental records describing the biology of *H. dermestoides*. In spite of this, several questions about the life cycle of the species still remain unanswered. The most important of these is the duration and number of larval instars and the duration of the life cycle. There are indications that the complete life cycle lasts one year. However, in Denmark, Thomsen *et al* . (1949) and Thomsen (1950) indicate the probability of a three-year duration of development. This was based on measurements of larval head capsules, and their observations in the forest confirmed that the development lasts at least two years and possibly three years. Fergusson (1920) indicates that the generation is normally an annual one. An outline of the major features of the life cycle of *H. dermestoides* is presented in this review.

2.1.4.1. Adult stage

Adult *H. dermestoides* have an elongate body 6 to 15mm long (Linssen, 1959), which in fresh specimens shows a yellow pubescence. The head is large and carries a pair of eleven-jointed serrate antennae, and the eyes are

prominent. The legs are slender and the seven-jointed abdomen is quite covered by the elytra. The most interesting point in its morphology is the marked sexual dimorphism. The male possesses a large, branched sense organ on the second joint of the large and flabellate maxillary palpi (Fergusson, 1920; Linssen, 1959); it can be extended by haemolymph pressure and serves as an olfactory organ (Jacobson, 1965). The male is (6 to 13mm) generally smaller than the female (9 to 16mm), but there are other distinctive differences. The male is proportionally narrower and the female broader; the elytra of the male are yellowish brown but black towards the apex (sometimes all black), where as in the female the elytra are yellowish brown and the thorax and eyes are black. There is considerable variation among the adults. Thomsen (1950) reports that as a rule, in the Danish race of *H. dermestoides* the male is all black, though brown males may be seen, the female is yellowish brown.

In Scotland adult *Hylecoetus* emerge in May and June. After pairing the females lay eggs in June and July. The female uses her protruding ovipositor to deposit eggs in bark crevices of stumps and dead trees. Eggs are also laid on stumps around the circumference on the exposed cambium ring. In these special cases the larvae on hatching tunnel downwards for several centimetres between the bark and the wood, afterwards making a separate wide gallery that pierces the bark for the removal of frass (Fergusson, 1920).

In Denmark *Hylecoetus* appear at the end of April or in May. The active life of the imagine lasts for only 2 to 3 days. Of the 56 adult *Hylecoetus* examined 39 were males, 17 females (♀:♂ sex ratio 1:2.3) (Thomsen *et al* ., 1949).

In Austria Kurir (1972) gives for emerged *Hylecoetus* a ♀:♂ sex ratio of 1:1.98, with an adult life span of 1 to 4 days: on average 2 days (males 2.16 days, females 1.5 days). During pairing the position of the copula is mostly

supra feminal. The male is active and they are able to copulate one to eight times. The copula lasts between 46 seconds and 36 minutes: on average 12 minutes and 25 seconds.

2.1.4.2. Egg stage

According to Thomsen *et al* . (1949), oviposition was observed in the laboratory on the day after emergence. Eggs are placed on uneven places on the bark, either singly or a few together. The incidence of foliose corticolous lichens may increase acceptable egg-laying sites (Thorpe, 1982). The egg is about 1.5mm in length, whitish, with an incubation period of about 7 days at a temperature of about 20°C. (Thomsen *et al* ., 1949). In Norway, *H. dermestoides* females were observed laying up to 108 eggs each in the laboratory. Oviposition occurred on the bark surface in late May as soon as the temperature exceeded 10°C. (Lyngnes, 1958). In Austria Kurir (1972) found that the cylindrical, light whitish-yellow 1.5 to 2.1mm long eggs were placed in heaps with a sticky secretion on smooth portions of the bark. Eggs laid singly or in pairs were rare, heaps of 4 to 91 eggs being common. The total production of eggs had a range of 99 to 146, with an average of 116 per female. The embryo developed in 7 to 9 days with a mean of 8 days.

2.1.4.3. Larva stage

The larva stage in *H. dermestoides* represents the longest single period of the life cycle, lasting from 1 to 3 years. This is also the only feeding stage. The first instar larva leaves its chorion upon hatching and then it feeds on the egg shell: a phenomenon of hunger, and through this feeding the spores of the ambrosia fungus reach the larval gut and are later deposited in the tunnel. The first stage larva is apparently negatively phototactic; it hides in crevices etc., and after 3 to 4 days bores into the bark forming a small hole of a diameter of 0.3 to 0.4mm. Later the larva continues into the wood and as it grows, it enlarges the tunnel; but the external opening remains the original size, making

it difficult to detect it except at the moment when bore-meal is thrown out.

The larva is whitish-yellow, elongate 15 to 24mm. The ninth abdominal segment has a characteristically rigid outgrowth which is used for ejecting the bore-meal through the hole in the bark (Sch neider-Orelli, 1920; Lyngnes, 1958). In the first instar larva the outgrowth is represented by a horny shield with two hooked spines. First instar larvae have 5 ocelli, which are lacking in later instars. Thomsen (1950) describes additional characters of the last larval instar, enabling this stage to be distinguished from earlier ones. The last instar larva has a brown and granular cuticle on the front of the head; a crescent-shaped cuticular ridge on the side of the head and behind this the imaginal eyes, visible through the semitransparent larval cuticle as a violet spot. The head is smooth and possesses strong gnawing mandibles, but no antennae are present. The three joints of the thorax are strong especially the first, which is much enlarged and arched. Each joint of the thorax carries a pair of legs. There are nine abdominal segments.

The larva does not have a definite pattern of tunnelling as in some scolytids. The tunnel is nearly horizontal, reaching 20cm; the diameter is almost the same in the whole length but may vary between 2 and 4mm in different cases. Fergusson (1920) found that in larch, the galleries were confined to the outermost ten annual rings, and the galleries which are somewhat curved or bow-like, followed the direction of the annual rings. Hayes (1987) also found that tunnels were confined to larch sapwood. In beech, the larva tunnelled for 7 to 12 inches (17.8 to 30.4mm) into and almost at right angles to the wood (Thomsen *et al* ., 1949). Galleries exposed by removing the bark are immediately plugged by the larva, but a narrow passage is left through which the tail may be seen projecting when bore-meal is being expelled.

Winter is passed in the larval condition, full size having not yet been

attained. In spring the larva resumes feeding and there is a renewal of the out throw of frass. When almost ready for pupation the larva turns in its tunnel, and makes its way towards the bark, often filling the gallery behind it with frass. Pupation takes place in that part of the gallery nearest the bark; in thick-barked material the pupal chamber may be entirely in the bark. The original entrance hole in the bark is enlarged by the adult beetle before it emerges (Fergusson, 1920). However, Thomsen (1950) reported that in spring the larva widens the external end of the tunnel at a length of 10 cm, and then it turns with its head against the surface. The external opening in the bark is enlarged to the full breadth of the tunnel, i.e. from 2.3 to 3.8 mm., and a plug of frass is made to close the outer end of the pupal chamber. Thereafter pupation occurs. There is no conclusive evidence about the duration and number of larval instars (Thomsen, 1950).

2.1.4.4. Pupa stage

The pupal stage lasts for about only 7 days, but the young beetle remains for a few days in the tunnel before it appears. The pupa measures up to 14mm. There are spines, the apices of which are turned forwards, on the upper surface of each segment of the abdomen. The spines aid the pupa in retreating down the tunnel if the gallery is exposed. The elytra, hind wings and legs are free i.e. the pupa is exarate.

2.1.5. Emergence, flight and host selection

In Scotland there is very little information about the emergence of *Hylecoetus* from the bark. Fergusson (1920) observed that at Dunkeld adult *Hylecoetus* emerge between May and June. After mate location and pairing, oviposition takes place in June and July. There is no information on the time of emergence, the seasonal distribution of the sex ratios of emerged *Hylecoetus* and causal factors for *Hylecoetus* emergence. The relationship between emergence and flight has not yet been established.

According to Fergusson (1920) *Hylecoetus* is on the wing from May to July. *Hylecoetus* has a four-day flight period which occurs in April-June (Eckstein, 1929a). In Britain, the species is a day flyer, with most flight taking place in the late evening around sunset (Linssen, 1959).

Thomsen *et al*. (1949) recorded that in Denmark *Hylecoetus* appeared during the last days of April or the beginning of May. No *Hylecoetus* were found in logs and window flight traps used in studies at two sites in southern Finland in 1972 (Löyttyniemi and Uusvaara, 1977) However, *Hylecoetus flabellicornis* were collected as indicated in Table 1. The flight season for *Hylecoetus* may be longer at lower latitudes than at high latitudes (Table 1).

Kurir (1972) found that in Austria, from 1968 to 1971, *Hylecoetus* was in flight from the 1st. April up to the 18th. May, with peak flight occurring between 11th. and 13th. April.

Table 1: RECORDS OF *Hylecoetus* CAUGHT IN FLIGHT.

COUNTRY	LOCATION	SPECIES	APRIL	MAY	JUNE	JULY	REFERENCE
AUSTRIA	47° 0'N, 14° 0'E	*H.d.*[1]	★	★	-	-	Kurir (1972)
GERMANY	54° 17'N, 10° 52'E	*H.d.*	★	★	★	-	Eckstein (1929a)
DENMARK	56° 0'N, 4° 0'E	*H.d.*	★	★	-	-	Thomsen *et al.*(1949)
SCOTLAND	57° 0'N, 10° 0'E	*H.d.*	-	★	★	★	Fergusson (1920)
FINLAND							Löyttyniemi
Bromarv	60° 02'N, 23° 02'E	*H.f.*[2]	-	★	★	★	and
Tusuula	60° 21'N, 24° 59'E	*H.f.*	-	-	★	★	Uusvaara (1977)

H.d.[1] = *H. dermestoides*; *H.f.*[2] = *H. flabellicornis*; ★ = *Hylecoetus* in flight.

2.1.5.1. Host selection: primary attraction

A general account of the type of material attacked by *H. dermestoides* is described by several workers (Bletchly and White, 1962; Kurir, 1972; Schwenke, 1974; Capeck, 1982 etc.). Trapping studies on *Hylecoetus* and *Trypodendron* using host logs (Bletchly and White, 1962; Löyttyniemi and Uusvaara, 1977), and the evidence obtained for other ambrosia beetles (Moeck, 1970; Cade, Hrutfiord and Gara, 1970), indicate that the mechanism used by *H. dermestoides* to recognize and colonize suitable hosts must involve a response of flying *Hylecoetus* to host-originating odours or attractants. The production of insect attractants by anaerobic fermentation in stumps, bolts, logs or diseased conifer trees and laboratory and field tests of their effectiveness in attracting beetles are described by Dethier (1947), Graham (1968), Moeck (1970) and Cade *et al* . (1970). Ethanol, a product of fermentation, alone or synergizes host metabolites (monoterpenes) to attract pioneer beetles in *Xyloterus lineatus* and *Gnathotrichus sulcatus* Le Conte (Moeck, 1970; Cade *et al* ., 1970). The role of monoterpenes, alone and in combination with ethanol, in the host selection of the scolytid *Tomicus piniperda* L. in pine (*Pinus sylvestris*) forests in the German Democratic Republic was studied by Vité, Volz, Paiva and Bakke (1986). Beetles were caught in window barrier traps baited with monoterpene hydrocarbons, (terpinolene and α-pinene contained in the oleoresin of *Pinus sylvestris*) and ethanol. *Hylurgops palliatus* was also caught in flight barrier traps baited with ethanol and terpinolene and/or α-pinene. *T. piniperda* was more attracted to α-pinene alone. Vité *et al* . (1986) concluded that colonization of pine stumps and logs by adults of *T. piniperda*, particularly by females, is in part due to the synergistic effect between monoterpenes (especially the host-specific terpinolene) and ethanol secondarily formed in the damaged wood tissue. Other examples of the response of beetles to ethanol and host monoterpenes are given by the following workers: Moeck (1971); Rolling and Kearby, (1975); McLean and Borden (1977); Borden, Lindgren and

Chong (1980); Montgomery and Wargo (1983); Dunn, Kimmerer and Nordin (1986). The response of *H. dermestoides* and some scolytid beetles *Ips typographus*, *Leperisinus varius* Fabricius, *Hylurgops palliatus* and *Tomicus piniperda*, and the xyleborins *Trypodendron lineatum* [*Xyloterus lineatus*], *Xyleborus dispar* Fabricius, *X. saxesen* Ratzeburg [*Xyleborinus saxesenl*] and *Xylosandrus germanus* (Blfd.) to flight barrier traps baited with their respective pheromones and/or ethanol was investigated in the field in the German Democratic Republic by Klimetzek, Köhler, Vité and Kohnle (1986). The response by all four ambrosia beetles and *Hylecoetus dermestoides* increased almost linearly with every tenfold step up of the ethanol concentration (Table 2); a similar reaction was first reported for *Trypodendron domesticum*. Klimetzek *et al* . (1986) indicated that ethanol perception represents a crucial mechanism in host allocation for many subcortical living insects, particularly ambrosia beetles which depend on the cultivation of fungal symbionts. As ethanol generates in sufficiently moist sapwood and phloem tissue only, it apparently signals suitable host material for fungal growth (ambrosia beetles), and host susceptibility as well (various *Hylesinae*). Accordingly, the role ethanol plays within a specific communication system appears to change with host preference. Klimetzek *et al* . (1986) suggested that a decreasing ethanol dependency coincides with an increasing specialisation in host selection: *ethanol represents the major signal:* "generalists" colonizing hardwood and conifers alike such as *H. dermestoides* or *X. germanus*; *ethanol synergizes host odours: H. palliatus*, *Hylurgops* and *Hylastes* spp (Wood, 1982); *ethanol synergizes host odours and/or attractant pheromones: Dyocoetes autographus*, *Trypodendron* spp., *Gnathotrichus* spp. *dosage-dependent synergism between ethanol attractant pheromones, and/or host odours:* this group ranges from pest species of low aggressiveness (*Leperisinus, Tomicus*), and possibly, the "turpentine beetles" (*Dendroctonus terebrans* [Oliv.] (Scolytidae), *Dendroctonus valens* Le Conte to major pest species such as the Douglas fir beetle

Dendroctonus pseudotsuga Hopk.; *ethanol-independent aggregation*: major bark beetle pests, such as *Dendroctonus frontalis* Zimmermann or *Ips typographus* attacking and overwhelming healthy host trees, in which ethanol generates only after successful attack. The pheromone response of some of these species seems, in fact, to be reduced by ethanol. It was concluded that high ethanol concentrations enhance the response of non-aggressive ("secondary") Scolytids and Xyleborins to aggregate on host trees, but interfere with the pheromone response among aggressive species. The study by Klimetzek *et al*. (1986) suggested that dosage response to ethanol deserves major consideration in future efforts at elucidating the evolution of aggregation patterns particularly among the "secondary" bark beetles.

Table 2: NUMBER OF BEETLES CAUGHT IN FLIGHT BARRIER TRAPS BAITED WITH DIFFERENT CONCENTRATIONS OF ETHANOL PER SE (3 REPLICATES EACH; / WEISWEIL, APRIL 5–25, 1985; // EHRENSTETTEN, MAY 4–JUNE 13, 1985). *X. dispar* (A), *X. saxeseni* (B), *X. germanus* (C), *H. dermestoides* (D).

	ETHANOL APPROX. RELEASE RATE [mg h^{-1}]	BEETLE SPECIES CAUGHT (A)	(B)	(C)	(D)
/	0.001	0^{a}	0^{n}	0^{n}	–
	0.01	0^{a}	2	0	–
	0.1	3^{a}	11	4	–
	1	41^{b}	24	14	–
//	0.5	1^{n}	0^{n}	3^{n}	10^{a}
	10	40	5	438	$38^{a.b}$
	25	52	11	615	$61^{a.b}$
	250	85	19	871	87^{b}

$^{a.b}$ Difference between treatments per experiment and the species significant at the p=0.05 level if marked with different letters; ^{n} not significant.

Source: Klimetzek *et al*. (1986).

2.1.5.2. Sex attraction

In other ambrosia beetles sex attraction is by use of aggregation pheromones. *Xyloterus lineatus* uses lineatin (3,3,7-trimethyl-2,9-dioxotricylo[3.3.$10^{4,7,}$]nonane), while *Gnathotrichus sulcatus* Le Conte uses sulcatol (6-methyl-5-hepten-2-ol). Laboratory tests of *H. dermestoides* have shown that the male detects the female from a distance by means of the maxillary palpi (Jacobson, 1965). If the maxillary palpi are removed, the male is no longer attracted by the female. Male *Hylecoetus* having their maxillary palpi coated with a film of gum mastic became inactive and would not mate with females in their vicinity. After the mastic was removed with alcohol they quickly became active and began to mate. The females on the other hand have a very simple type of maxillary palpi and antennae. The male detects the female with the maxillary palpi which are well supplied with nerve stalks. This work demonstrated that female *H. dermestoides* produces a pheromone which attracts male *Hylecoetus* for mating (Jacobson, 1965). Klimetzek *et al* . (1986) noted that *H. dermestoides* seem to lack aggregating pheromones. However, Francke *et al* . (1984) working in the German Democratic Republic isolated a cyclic enolether 2,5-dimethyl-2-isopropanol-2,3-dihydrofuran and concluded that the compound is sex-specific and appeared to be a component of the female sex pheromone. This is the first record of cyclic enolethers to be identified from insects, and include 2,6-diethyl-3,4-dihydro-3,5-dimethyl-2,*H*-pyran (anhydro serricornia) from *Lasioderma serricorne* (F.) (Anobiidae).

2.2. Nutrition of *H. dermestoides*

Insect nutrition as defined by House (1961) includes nutritional requirements (chemical factors essential to the adequacy of the ingested diet), chemical feeding requirements (chemical factors important to normal feeding behaviour) and physical feeding requirements (dietary texture, position, light

intensity and other physical feeding factors influencing feeding behaviour).

2.2.1. Insect–fungus symbiosis

Although *H. dermestoides* larvae bore into wood the whole feeding strategy is based upon the fact that *H. dermestoides* is associated with an ambrosia fungus. Therefore the ability of *H. dermestoides* to exploit potential food resources is dependent upon its symbiotic association with microorganisms. While the majority of ambrosia beetles are in the families Scolytidae (some) and Platypodidae (all) as indicated by Bletchly and White (1962); Batra (1967) etc. Chapman (1978), reported that all lymexylids have ambrosia fungal associations. The coexistence of ambrosia beetles and their fungi is a typically mutualistic ectosymbiosis i.e. the cultivation of certain fungi outside the insect body in wood as a medium. This feeding habit is known as xylo–mycetophagy. The insects are wood borers but not wood–feeders, the symbiotic fungi which grow on their tunnel walls acting as their primary food source (Crowson, 1981). In some Scolytidae and in the Platypodidae the adult beetles are usually xylomycetophagous. Ambrosia fungi appear in the tunnel systems shortly after the infestation of a host tree by the beetles where they form a palisade–like mat or a cushion of conidiophores and are rich in nutrients. The conidia generally appear in monilioid chains, but in other cases singly, or crowded at the apex of or on the sides of simple conidiophores.

2.2.1.1. Role of fungus in symbiosis

Exact details of the symbiosis, particularly those of fungal and beetle nutrition, are not fully understood. However, it is generally held that the beetle derives a major part of its nutritional requirements from eating the fungus. Cooke and Rayner (1984) point out that the basis for mutualism is that the insects do not themselves produce the enzymes necessary for digestion of wood. Successful colonization is therefore dependent on the fungi which degrade wood and render the wood digestible, or act as a source of

wood-degrading enzymes which can be acquired by the insects, or the fungi can themselves be utilized as a major food source. The fungi may additionally supply the insect with essential compounds, for instance amino acids, vitamins and sterols, in which wood is normally deficient. Other workers (Kok, Norris and Chu, 1970); Norris, 1972; Kok, 1979) have shown that the fungal lining on the walls not only provides ambrosia beetles with most of their gross nutrients, but also those amino acids that are essential for fertility in adults, together with ergosterol, which is involved in both adult fertility and larval pupation.

In addition to providing nutrients in the broad sense, ambrosia fungi may supply compounds that control normal development. An indication of the complex nature of the association may be obtained by examining the association between *Xyleborus ferrugineus* (F.) (Scolytidae) and its ambrosial associate. Adults of *X. ferrugineus* have been reared from aseptic eggs on complex agar media containing sucrose, protein and yeast extract, together with plant extracts rich in lipids, polysaccharides and vitamins, etc., (Saunders and Knoke, 1967). Females tunnel and feed within the medium, but eggs produced by these asymbiotic adults are not viable. Oviposition of viable eggs takes place only after a mutualistic fungus *Fusarium solani* (Mart.) Appel et Wollenweber is inoculated into the medium (Norris and Baker, 1967). This fungus is normally present in the oral mycangia (see Page 31) of symbiotic adults. The presence of the ambrosia fungus, or rather of specific chemicals it produces, is essential for reproduction but not for growth. It has been found (Norris and Baker, 1969a; Norris, 1972; Kok, 1979) that fungus-free females supplied with cholesterol produce second-generation larvae, which do not pupate. However, *Fusarium solani* also produces ergosterol, and the supply of exogenous ergosterol to asymbiotic beetles allows growth, development and reproduction to proceed normally (Kok *et al* ., 1970). Maternal aposymbiotic females of *Xyleborus ferrugineus* lost their ability to pass required pupation

factors to their progeny, and initial reproduction of females maintained on a fungus diet was significantly earlier than for those beetles kept on a non-fungus diet (Kingsolver and Norris, 1970). Norris (1972) obtained evidence that the *X. ferrugineus* female adult can pass, apparently transovarily, to her asexual progeny sterol or sterol-dependent metabolites necessary for pupation. Supply of the fungus *Ambrosiella hartigii* Batra to post diapause females of the ambrosia beetle *Xyleborus dispar* (Fabricius) allows oocyte development and oviposition (French and Roeper, 1975), and larvae require the fungus for normal development and pupation (French and Roeper, 1972, 1973). Fungal symbiotes also provide *Xyleborus* beetles with essential amino acids (e.g. lysine, methionine, arginine and histidine) required for the initiation of reproductive processes. A *Cephalosporium* and a *Graphium* species have been isolated from the mycetangia of *X. ferrugineus* and these too, when fed to adults or larvae, allow development to take place but are not so beneficial as *F. solani* (Baker and Norris, 1968). Cooke (1977) suggests that the insect benefits from the mutualistic association because: (1) the fungus weakens the wood elements by its growth for 8 to 15 days prior to the emergence of larvae thus facilitating excavation of cells occupied by larvae and pupae; and (2) the larvae are exclusively mycetophagous and thus derive all their nutrition from the fungus: also callow adult beetles, e.g. *Xyloterinus politus* (Say) (MacLean and Giese, 1967; Haanstad and Norris, 1985), *Monarthrum mali* (Fitch), *M. fasciatum* (Say) and *Xyleborus* spp. feed on the microorganismal growth lining the cradle walls. In the Lymexylids the fungi are of importance only in larval nutrition. Kelsey (1958) found that *Anobium punctatum* (De Geer), did not grow normally without their yeast symbiote. This showed that the separation of insect and fungus may lead to disturbances in the development of the insects. Batra and Michie (1963) state that the larvae of ambrosia beetles utilize ambrosia as the only source of food. The ambrosia supplies the insect with a highly nitrogenous, low residue diet synthesized by using nutrients located far away from the larvae. It

re-utilizes part of the nitrogen excreted by the beetle for further growth, and it indirectly shifts the carbon and nitrogen of the wood from the inner layers of wood towards the surface in the form of insect frass.

2.2.1.2. Role of insect and mycangia in symbiosis

One problem in any microbe-animal association concerns the way in which the microbe is transmitted from individual to individual. In insects the transmission of fungal material in a viable condition, usually in the form of spores, is ensured by such means as simple adherence to the exoskeleton or ovipositor or else by the development of special organs (mycangia or mycetangia) which contain viable fungal material.

In the Scolytidae, transport involves the use of various types of pits, recesses or invaginations in the outer integument which function as "mycangia" (Batra, 1963a). Such cavities are usually located on the body surface or the mandibles but rarely on the other appendages. Frequently mycangia are associated with the basal articulate of appendages or with intersegmental functions and it has been suggested that this might make it possible for the cavities to be voided of their contents by body movements. Mycangia are often, but not always, confined to the females and represented only by vestiges in the males. The location of mycangia on the body and their range of structure vary but are constant for any single species of coleoptera. These invaginations may secrete an oily liquid from a lining of secretory epithelium, in which the fungus is maintained in a toruloid condition. Where mycetangia are sac-like they may have heavy resculature. Batra (1967) indicated that only the primary and not the auxiliary fungi are found in these organs. Mycetangia fungi may also be present as spores and retained within the organs by means of hairs or spores that project from the host integument.

In the lymexylids propagules of the ambrosia fungus are carried in the

grooves in the ovipositor in *Hylecoetus* and *Melittomma*. Adult female Lymexylids deposit their eggs under the bark of dead or dying trees and stumps, and during oviposition inoculate the eggs with specific fungi. Batra and Francke-Grosmann (1961) reported that the ambrosia fungus for *H. dermestoides* and *H. lugubris* is disseminated by its insect partner during oviposition. Baker (1963) and Burnett (1976) reported that dispersal of fungi which grow in galleries made by various kinds of boring insects is fortuitous and varies through the spores being swallowed or adhering in some way to the insect.

Francke-Grosmann (1967) has reviewed the symbiosis in *H. dermestoides*, and reported that in *Hylecoetus* certain pouches situated at the end of the ovipositor serve as organs of fungus storage and fungus transmission. At the ventral side of the long, telescope-like retractable ovipositor, close to the opening of the oviduct, a pair of integumental folds are formed at either side of a median groove, and both (the pouch and the groove) are filled with very tiny spores of the fungal symbiote (Figs: 5 and 6). The fungus is transferred to the new generation by contaminating the eggs during oviposition, while the egg passes close to the groove and the folds. Moreover, there are some muscles present, the contraction of which may contribute to squeezing out the contents of the pockets. The spores are embedded in a slimy substance which makes them adhere to the surface of the egg. The way they are introduced into the new tunnels by the young larvae was described by Kurir (1972) who pointed out that the first instar larva feeds on its chorion; the latter is contaminated with fungal spores and in this way the spores get into the tunnels. The fungus symbiont has been isolated from the excremental traces of young larvae on agar plates. Specialization in morphology, biology and behaviour of *H. dermestoides* promotes the symbiosis with its ambrosia fungus: the host selection of the mother beetle i.e. choosing diseased trees,

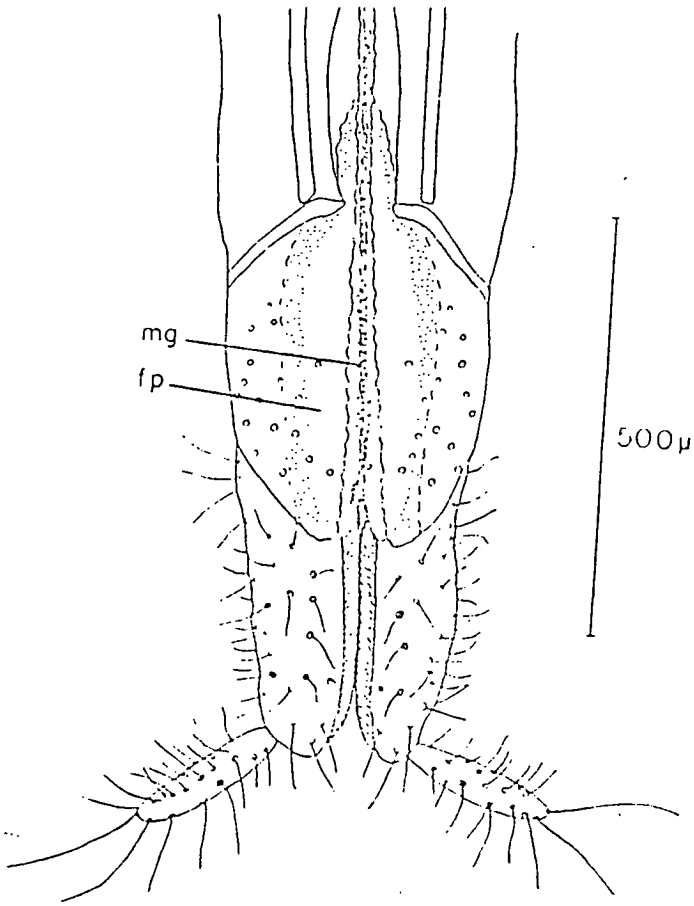


Fig:5 End of ovipositor of *H. dermestoides* (ventral view) (fp, fungal pouches shining through the integument; mg, median grove). (Source: Francke-Grosmann, 1967).

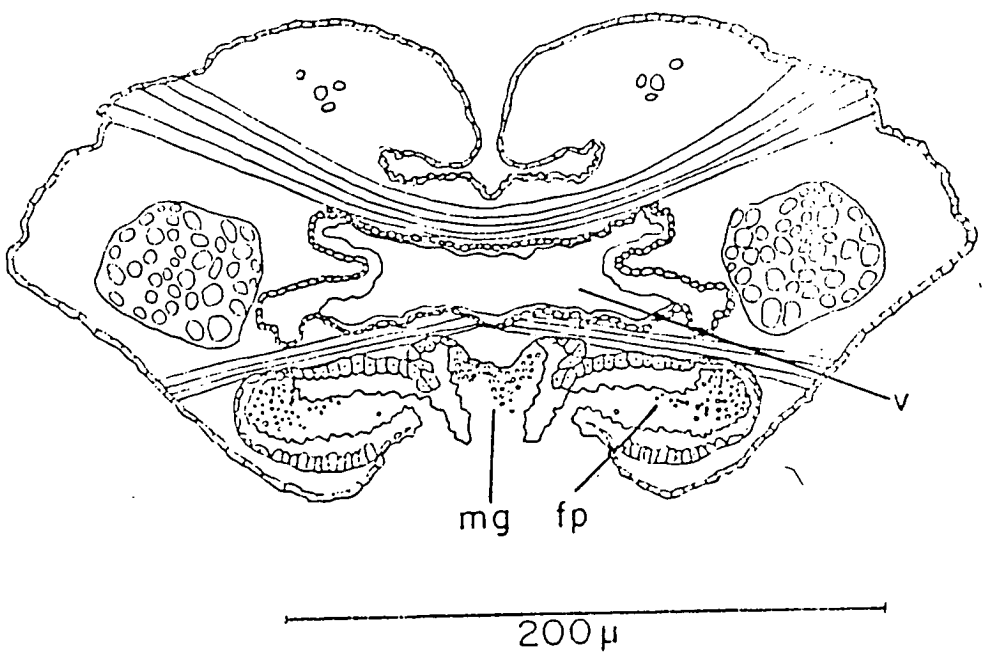


Fig:6 Cross-section through end of ovipositor of *H. dermestoides* (v, vaginal; fp, fungal pouches; mg, median grove). (Source: Francke-Grosmann, 1967).

stumps and trunks with a relatively low moisture content for egg-laying (Schneider-Orelli, 1920; Rummukainen, 1947; Thomsen *et al* ., 1949; Thomsen, 1950; Thorpe, 1982), the vaginal pouches, the feeding of the first larva on its chorion and the habit of the larva in keeping its tunnel clean by pushing out the bore-meal ensure the transmission of the fungus into the new larval dwelling.

In *Dendroctonus frontalis* mycetangia secretions are produced by two types of glandular cells, and it has been suggested that such secretions serve as regulators of the growth of ambrosial fungi within the mycetangia and also act as inhibitors of the growth of non-ambrosial fungi. During boring, secretion increases so that the oily liquid oozes out of the mycetangia carrying fungal cells with it. This process is aided by contraction of the muscles and movement of the mycetangial hairs. The fungi then begin to grow in the tunnels, perhaps initially utilizing the secretions smeared on the walls. How ambrosia fungi enter the mycetangia from the tunnel wall after pupation has taken place is not known.

Although degradation of wood provides the bulk of nutrients for ambrosial growth, excreted host-metabolites which are principally nitrogen compounds may contribute to fungal nutrition, and in addition, may have morphogenetic effects. For example, urates, the major nitrogenous excretory products of Xyleborine beetles, probably induce and maintain the typical monilioid development of ambrosia fungi. Ambrosia fungal growth and monilioid development seem to be largely dependent on the activity of adult females within the tunnel systems. In addition, activity of these brood females, and possibly also of larvae, appears to reduce or inhibit growth of other saprotrophic fungi which might otherwise compete with ambrosia species. Exactly how much inhibition is maintained is not known, but after progeny have left the tunnels and the brood females have died ambrosia species are quickly

overgrown and eliminated by alien fungi, indicating that they are non-combative. If the beetles release anti-fungal metabolites, as seems likely, then the fungi may be regarded as stress tolerant. Certainly stress tolerance seems to obtain within mycetangial fluid, yeast-like growth probably being a result of the generally unfavourable conditions for fungal development. Also lack of ruderal qualities may further enhance dependence on the insects since the phase of ambrosia development in wood may be brief: for instance, the time from tunnelling and egg-laying until the brood galleries are abandoned can be as short as 30 days (Cooke and Rayner, 1984).

The inoculum of many ambrosia fungi removed from tunnels fails to germinate in cultures. Although the fungi taken from mycangia will germinate, some ambrosia fungi from either the tunnels or the mycangia will not germinate in the absence of adult beetles (Batra and Michie, 1963).

Batra (1963a, 1967) reported that the fungus benefits from the insect because: (1) it is transmitted and inoculated directly into the wood having suitable moisture content and nutrients; (2) injury to wood elements by the beetles (Platypodidae and Scolytidae) allows rapid penetration of hyphae; (3) urea and uric acid present in the frass serve as an important source of nitrogen; (4) the fungus, which is normally very susceptible to desiccation, receives protection in mycangia during the flight and hibernation of adult beetles; and (5) the fungus inoculum multiplies in mycangia and produces a large number of reproductive units called ambrosia cells. Roeper and French (1981) studied the growth of the ambrosia fungus *Ambrosiella hartigii* on various nitrogen sources in artificial culture, and found that the fungus (1) grew significantly greater on soluble amino acid-nitrogen sources than other nitrogen sources; (2) the fungus appeared to be nonproteolytic; and (3) the fungus did not utilize possible insect excretory products such as allantoin or uric acid . Growth on ammonium-nitrogen varied according to pH.

2.2.1.3. Taxonomy of ambrosia fungi

Many fungi are regarded as being ambrosial associates, including species in the genera:*Absidia* van Tieghem, *Ambrosiella* von Arx and Hennerbert, *Ambrosiamyces* Trotter, *Ascoidea* Brefeld and Lindau, *Aspergillus* Mich. ex Fries, *Bostryodiplodia* Sacc., *Candida* Berkhout, *Cephalosporium* auct. non Corda, *Ceratocystis* Ellis and Halst, *Chaetomium* Kunze ex Fries, *Cladosporium* Link ex Fries, *Colletotrichum* Corda Corda, *Endomyces* Reess, *Endomycopsis* Dekker, *Fusarium* Link ex Fries, *Graphium* Corda, *Leptographium* Lagerberg and Melin, *Monacrosporium* Oud., *Monilia* Pers. ex Fries, *Mortierella* Coemans, *Paecilomyces* Bainier, *Penicillium* Link ex Fries, *Pestalozzia* de Not, *Phialophoropsis* Batra, *Raffaela* v. Arx and Hennebert, *Sporothrix* Hektonen and Perkins, *Sporotrichum* Link ex Fries and *Tubercullariella* Tode (Batra and Francke-Grosmann, 1961; Batra, 1963a; Batra and Francke-Grosmann, 1964; Batra, 1967; Baker and Norris, 1968; Kok, 1979). Some of the fungi are primary ambrosia fungi, whereas others are secondary. Secondary or auxiliary ambrosia fungi provide ambrosia beetles with supplementary nutrients (Norris, 1979b). Francke-Grosmann (1967) stated that auxiliary ambrosia fungi often appear in the galleries during the pupal stage and are eaten by emerged adults. Blue-stain fungi, *Ceratocystis*, *Botryodiplodia* and *Leptographium* may be primary or auxiliary ambrosia fungi.

2.2.1.4. Specificity of ambrosia fungus-beetle symbiosis

Buchanan (1941) reared larvae of *Xylosandrus germanus* to the adult stage on non ambrosia fungi, *Ceratocystis ulmi, C. pluriannulata* (Hedge) C. Moreau, and a *Pestalozzia* spp. Francke-Grosmann (1967) alluded to Buchanan's work and noted that at least some beetles do not have a specific fungus. Cooke (1977) noted that some confusion exists concerning the question of the possible specificity of fungus-beetle associations. He noted that each beetle species is associated with a specific fungus, or complex of fungi.

Crowson (1981) extended Cooke's hypothesis, indicating that closely related taxonomic groups of beetles [species] may be associated with one fungus or different strains of the same fungus or closely related fungi, or else that the fungus is specific to the host plant and not the beetle. It has been pointed out that whatever form the symbiosis may take, it is evidently not complete as at least some ambrosia fungi can occur freely in nature without association with the beetles (Bakshi, 1950). More recently, Haanstad and Norris (1985), noted that the microorganismal growth occurring on the walls of the ambrosia beetle *Xyleborus dispar*'s gallery was fungal, most workers have stated or implied that each ambrosia beetle is symbiotically asociated with one species of microbe upon which it relies for nutrition. However, Haanstad and Norris (1985), have added that some investigators have variously reported that ambrosia beetles have more than one associated microbe. Such additional microbes have included yeasts, yeastlike fungi, and bacteria in addition to filamentous microfungi. Ambrosia beetles also may have associated mites (Browne, 1961), nematodes (Verrall, 1943), and other parasites and predators (Browne, 1961). Norris (1965, 1979a,b) termed such an assemblage of symbiotic micro- and macroorganisms (including the beetle) a multi complex or a supraspecies. Norris (1965) hypothesized that it is the activities of the microbial complex as a whole, rather than just of an ambrosia fungus, that allow such beetles to indirectly utilize nutrient-poor substrates such as wood. Abrahamson and Norris (1969) had studied the ectosymbiotic filamentous fungal components of *Xyloterinus politus*-associated community. In those studies, yeasts, yeastlike fungi and bacteria were also noted, but such microorganisms were not charactierized. Haanstad and Norris (1985) have extensively studied the microorganisms variously associated with *Xyloterinus politus* as it attacks black oak *Quercus velutina* Lam. The results obtained by the latter workers, are summarized in Table 3. These results support the hypothesis that several microbes i.e. yeasts, a yeastlike fungus, filamentous microfungi and bacteria,

are intimately associated with this ambrosia beetle in its brood gallery. Haanstad and Norris (1985), therefore, concluded that a complex of several microbes, not just one ambrosia fungus, is intimately and symbiotically associated with this species of ambrosia beetle. Such findings provide new bases for the development of more general hypotheses concerning ambrosia beetle–microbial associations.

Table 3: SITES IN *Xyloterus politus* AND GALLERIES FROM WHICH YEASTS, YEAST LIKE FUNGUS, AND BACTERIA WERE COMMONLY ISOLATED

	BEETLE ISOLATIONS			GALLERY ISOLATIONS		
Genus	Head	Thorax	Abdomen	Cradle wood and plug	Gallery wood	Wood near gallery
Yeasts						
Candida	X	X	X	X	X	X
Pichia	X	X	X	X	X	X
Yeast like fugus						
Sacharo mycopsis	X			X	X	X
Bacteria						
Flavobacterium			X		X	X
Alcoligenes			X		X	X
Gluconobacter 'A'			X	X		
Gluconobacter 'B'	X	X	X	X	X	X

Source: Haanstad and Norris (1985).

Yeasts and the yeastlike fungus associated with *Xyloterinus politus* and their galleries have been previously isolated from scolytid or platypodid beetles (e.g., Baker, 1963; Norris, 1965; Kabir and Giese, 1966; Batra, 1971). Among the three bacteria reported (Table 3) as ectosymbiotes of *X. politus, Alcaligenes* and *Flavobacterium* were previously reported as associated with a scolytid beetle. *Gluconobacter* has been previously isolated from honey bees.

The frequent failure of past studies of these associations to report more

than one consistently isolated microbial ectosymbiote perhaps is attributable to inadequate techniques. In this regard, the growth of ambrosial fungi is usually very evident, even to the naked eye, and so it is usually readily isolated. Other types of microbes, now shown to be intimate ectosymbiotes of *X. politus*, may be consistently less obvious to the observer, and such relative obscurity of these ectosymbiotes may be accompanied by significant isolation difficulties. However, such difficulties are not reasonable bases for relegating these ectosymbiotes to the status of contaminants, as has historically been the usual interpretation.

Haanstad and Norris (1985) added that the association of a multispecies complex of ectosymbiotic microorganisms with a species of ambrosia beetle has several ecological implications. The consistent presence of several ectosymbiotes implies that each has it own ecological niche in the symbiotic community. Even though such niches may overlap, each member of such a symbiotic complex apparently may achieve a unique role in the community under conditions that are free of competition. Such ectosymbiotes of *X. politus* most likely have co-evolved mutualistically with this beetle over many centuries. Such co-evolution commonly yields reduced antagonistic (competitive) interactions and accentuated synergistic (cooperative) interrelationships, this confirms the interpretations of Norris (1965, 1979a,b).

The apparent major mutual goal of all species in such a symbiotic complex, including the ambrosia beetle, is to derive, directly or indirectly, nutrition from the available woody substrate. Even though *in vitro* studies have documented that single species of microbes can degrade lignocellulose, such pure cultures do not prevail in nature. The demonstrated presence of several diverse microbes in situations where lignocellulose transformation occurs naturally strongly supports the involvement of multispecies complexes in such degradation. Thus, it should be expected that multispecies complexes of

microbes are associated symbiotically with ambrosia beetles in their efforts to derive nutrition indirectly from wood.

Under experimental conditions, Baker and Norris (1968) have shown that the microbial mass of single species can meet the nutritional requirements of an ambrosia beetle, but these microbes grow consistently in mixed cultures in nature, not as pure cultures. Thus, the co-evolved mutualistic microbial complex is what constitutes the natural food for the beetles.

H. dermestoides was common in 1926 in Germany in beech, oak and spruce stumps and larval tunnels contained an ambrosia fungus on which the larvae fed. The abundance of *H. dermestoides* was regarded as being due to an increase of the ambrosia fungus in rainy years, and the decrease in numbers of the insect as being due to the abrupt scarcity of the fungus in dry years (Werner, 1929). A symbiosis also seems to exist between the lymexylid *Melittomma insulare*, and a yeast-bacteria complex (Brown, 1954). In Norway, *H. dermestoides* larvae were observed feeding on *Ascoidea hylecoeti* growing on gallery walls (Lyngnes, 1958). The former worker also found other fungi *Isaria* sp., *Aspergillus* sp. and *Verticillium* sp. in *Hylecoetus* larval tunnels, and spores of *Verticillium* were observed adhering to the maxillary palpi of male beetles. Female beetles were also noted by Lyngnes (1958) as playing an important role in the dissemination of the fungi. In Germany *Ascoidea hylecoeti* Batra and Francke-Grosmann was isolated from tunnels *Hylecoetus* in *Pinus* spp., and the fungus is described as the ambrosia fungus for the beetles (Batra and Francke-Grosmann, 1961). In Sweden, *A. hylecoeti* is established as the ambrosia fungus for *Hylecoetus* (Batra and Francke-Grosmann, 1961). The American *Hylecoetus lugubris* is associated with a different strain of *A. hylecoeti* as its ambrosia fungus (Batra and Francke-Grosmann, 1961). Another *Ascoidea*, *A. africana* sp. n. was isolated from the tunnels of an unidentified tropical lymexylid (Batra and Francke-Grosmann, 1964). According

to Batra and Francke-Grosmann (1961, 1964) all Lymexylid ambrosia fungi studied are species of the hemiascomycetous *Ascoidea*.

2.2.1.5. Nutritional composition of wood

The main components of woody tissue are lignin and cellulose. Cooke and Rayner (1984) noted that readily accessible, assimilable substrates such as starch, soluble sugars, lipids, peptides and other primary metabolites occur in relatively small amounts in wood (<10% by dry weight), being found almost exclusively in living or recently dead sapwood parenchyma. Their amount varies according to seasonal and other factors, but may be as high as 7 percent for starch and 2.5 percent for lipids with the amount of starch sometimes being maximally in the middle sapwood, declining to zero at the heartwood boundary. In European larch (*Larix decidua*), the main host for *H. dermestoides* at Dunkeld, Balogun (1969) found four sugars: raffinose, sucrose, glucose and fructose, present in the bark.

A three-fold reduction in nitrogen content from the first to the sixteenth annual ring in the sapwood of a *Populus* stem was correlated with a two fold reduction in decay susceptibility (Merill and Cowling, 1965). Much of the available nitrogen in wood is organic, mainly present as amino acids. More nitrogen is located in sapwood than heartwood; in recent sapwood than in older sapwood (Table 4); in early wood than in late wood of an annual ring (Table 5); in pith than in adjacent tissues; in inner than outer heartwood where the proportion of parenchyma cells is highest; and in root than stem wood of gymnosperms (Merill and Cowling, 1966). In European larch bark tissue the principal amino acids are valine, leucine, arginine, threonine, histidine, glutamic acid, aspartic acid, serine, α-alanine and glycine (Balogun, 1969).

Table 4: GROSS DISTRIBUTION OF NITROGEN AND NITROGEN CONTENT OF VARIOUS TISSUES IN SECTIONS OF TREE STEMS

Type of tissue	Spruce	White pine (tree No.3)	Ash (Tree No.1)	Oak
Gross distribution of nitrogen, % of total nitrogen in each cross section				
Heartwood	17.8	11.0	27.1	21.6
Sapwood	28.2	29.3	31.0	37.8
Whole wood	46.0	40.3	58.6	59.4
Inner bark	38.7	56.4	21.8	33.8
Outer bark	15.3	3.3	19.6	6.8
Whole bark	54.0	59.7	41.4	40.6
Nitrogen content,% dry weight of tissue				
Heartwood	0.06	0.05	0.09	0.08
Sapwood	0.09	0.09	0.11	0.20
Whole wood	0.07	0.07	0.10	0.12
Inner bark	0.58	0.50	0.31	0.31
Outer bark	0.54	0.24	0.45	0.59
Whole bark	0.57	0.47	0.36	0.33
Whole cross section	0.14	0.14	0.14	0.17

Source: Merill and Cowling (1966).

Table 5: PERCENTAGE NITROGEN CONTENT OF EARLYWOOD AND LATEWOOD IN SAPWOOD OF EASTERN WHITE PINE AND WHITE ASH

Eastern white pine				White ash			
Annual increment*	Early wood	Late wood	Early wood: late wood ratio	Annual increment*	Early wood	Late wood	Early wood: late wood ratio
2	0.066	0.059	1.12	4	0.138	0.080	1.72
4	0.061	0.045	1.35	5	0.143	0.078	1.83
5	0.051	0.048	1.06	6	0.128	0.073	1.75
6	0.055	0.045	1.22	7	0.125	0.063	1.98
7	0.054	0.046	1.17	8	0.137	0.077	1.78
9	0.036	0.031	1.16	9	0.134	0.081	1.65
10	0.043	0.037	1.16	10	0.132	0.075	1.76
11	0.041	0.034	1.21	11	0.135	0.076	1.78
13	0.043	0.042	1.02	12	0.132	0.081	1.69
14	0.039	0.028	1.40	13	0.137	0.074	1.85
Mean	0.049	0.042	1.17	Mean	0.134	0.076	1.77

* = measured from the cambium; Source: Merill and Cowling (1966).

Metallic elements essential for all fungi include phosphorus, sulphur, potassium, magnesium, iron, zinc, manganese, copper and molybdenum. Some fungi require calcium (Lilly, 1965). These important mineral nutrients are found in wood. Phosphorus and potassium show a decrease in concentration by as much as 95 percent or more during the transition from sapwood. The effects of such changes on fungal growth do not appear to have been investigated (Cooke and Rayner, 1984).

2.2.1.6. Ambrosia beetle nutrition.

Although wood is a food source for many insects, it is a particularly uncompromising medium for insects in that its main components, lignin and cellulose can not be digested by insect enzymes. In addition, woody substrates are deficient in sterols and vitamins compounds which insects require but can not synthesize. Ambrosia beetles obtain their nutrient requirement indirectly through their fungus symbiotes (Kok *et al* ., 1970). Wood fragments are always present in alimentary canals of feeding ambrosia beetles and larvae. However, it is not known whether this is an important factor in their diet, although its starch content is depleted during its passage through the digestive tract (Baker, 1963).

2.2.1.7. Growth of ambrosia fungi

The nutritional requirements and physiological properties of ambrosia fungi have not been rigorously studied. Species seem to differ from one another in their physiology and, apart from their ability to digest one or more components of wood, seem to have few physiological characteristics in common (Cooke, 1977). Glucose was found to be the major carbon source for ambrosia fungi, and certain strains of ambrosia fungi may use cellulose as a carbon source. Among taxonomically related fungi, *Endomycopsis platypodis* sp.n., in axenic culture, will utilize nitrate as a sole nitrogen source, while the congeneric species *E. fasciculata* Batra will not do so (Batra, 1963b). Some

ambrosia fungi will utilize uric acid as a nitrogen source and others will not. Batra (1963b) suggested that *E. platypodis* may be able to utilize uric acid as a nitrogen source. Urates (major nitrogenous excretory products of *Xyleborus*), urea or aspartic acid cause monilioid growth by *Fusarium solani* in agar media at initial pH between 6 and 7. *Monilia ferruginea* Mathiesen-Käärik, the ambrosia fungus of *Xyloterus lineatus*, requires casein, peptone or olive oil for successful spore germination on agar media, and will produce sporodochia only in the presence of these complex nutrients (Baker, 1963).

Some species of ambrosia fungi are heterotrophic for vitamins, while others are autotrophic (Baker, 1963).

While present in the mycangia ambrosia fungi may be exposed to particular and complex nutritional regimes. Thus, *Xyleborus* mycangia contain fatty acids, phospholipids, free sterols, sterol esters and triglycerides and large amounts of free amino acids, proline, alanine and valine. Moderate amounts of free arginine, histidine and aspartic acid are also found in the mycangia of these beetles (Abrahamson and Norris, 1970). Proline and glutamic acid as major nitrogen sources cause symbiotic fungi to grow as propagules in agar media at neutral pH. However, fungal growth is predominantly mycelial on acid media (e.g. pH 5.8, 4.8 and 4.4) to which proline and glutamic acid have been added. Norris (1979b) concluded from this work that the propagule forms of symbiotic fungi in mycangia of *Xyleborus ferrugineus* seem attributable to free proline in the insect's haemolymph and body secretions; and to a pH near neutrality in the mycangia. This molecular and ionic situation provides the beetle with the ability to keep its mycangial-borne symbiotes in relatively slow-growing and non parasitic states. Norris (1979b) suggested that the free amino acid pool, especially L-proline, of the haemolymph and body secretion from the beetles was the major nutrient cause of the growth of the symbiotic fungi in the mycangium of *Xyleborus dispar* Fabricius. French and Roeper (1975)

had previously reported that soluble amino acid-nitrogen increased significantly in the bodies of *Xyleborus dispar* in the spring when the beetles were in flight and initiating their brood systems. At this time, the ambrosia fungus *Ambrosiella hartigii* was found proliferating in and from the mycangium of *X. dispar*.

In old galleries of *Hylecoetus* the ambrosia fungus growth is regressive and the walls of tunnels with full-grown larvae become dark-stained and many secondary or auxiliary fungi such as *Ceratocystis* and *Leptographium* sp. or *Isaria*, *Aspergillus* and *Verticillium* sp. appear (Lyngnes, 1958). Fragments of the ambrosia fungi and secondary fungi were constantly found in larval guts (Francke-Grosmann, 1967).

The nature of any nutritional symbiosis between *Ascoidea* fungi and Lymexylid beetles remains obscure. In addition, the means by which the Scottish *Hylecoetus* nutritionally utilizes its woody substrate are unknown. However, in Germany the ambrosia fungus for *Hylecoetus* was found feeding on xylem sap and could possibly utilize the contents of parenchymatous cells in wood and inner bark (Francke-Grosmann, 1967). It has not been determined whether the components of the cell walls are decomposed or not. However, there is some influence on the middle lamella since the wood showed traces of maceration near the tunnel walls where the conducting vessels of the wood were filled with masses of hyphae. It was pointed out by Francke-Grosmann (1967) that the *Hylecoetus* ambrosia fungus provides the insect with a substantial food, rich in proteins, lipoids and glycosides. The hyphae of the fungus do not penetrate deeply into the wood, and he found a positive iodoform reaction when adding iodine and KOH to the fluid obtained by distilling the culture liquid; he concluded that, presumably, the glucose of the medium had been turned into alcohol by the fermentative activity of the fungus.

It may be added that the assocation between *H. dermestoides* and its fungus in most cases includes a third partner, a mite of the family Tryglyphidae; the deutonymph of this species was described as *Histiogaster hylecoeti* n.sp. (Acarien:Tyroglyphidae). The deutonymph are the migratory stages of a symphorisic mite of *Hylecoetus dermestoides*, that they are carried under the elytra of the females and leave them during their oviposition.

2.2.2. Ambrosia beetle–blue stain fungi symbiosis

Bakshi (1950) found that only blue-stain fungi were associated with ambrosia beetles in Britain. At Blair Atholl, *Ceratocystis piceae* and *Leptographium lundbergii* Lagerberg and Melin were isolated from tunnels of *Xyloterus lineatus* in standing dying Japanese larches. *H. dermestoides* and the bark beetles *Pityogenes chalcographus* and *Dryocoetes autographus* had attacked the same trees. At Dunkeld, *C. piceae* and *L. lundbergii* were isolated from felled logs of Norway spruce (*Picea abies*) attacked only by *X. lineatus*. In the same area *Oedocephalum lineatum* Bakshi and *C. piceae* were isolated from Japanese larch logs attacked by *X. lineatus*, *P. chalcographus* and *D. autographus*, and *Ceratocystis ambrosia* Bakshi was isolated from tunnels of *Xyloterus domesticus* in birch logs.

Leptographium lundbergii, *C. piceae* and *O. lineatum* were in addition isolated from alimentary canals of adults and larvae of *X. lineatus* by Bakshi (1950). Feeding experiments on the beetles to see whether the larvae could be brought from egg to pupation on cultures of their respective fungi were not carried out. However, as the fungi were constantly isolated from the galleries of the ambrosia beetles from different localities in pure culture, Bakshi (1950) believed that they should be considered as 'ambrosia' in the true sense. Further, Bakshi (1950) noted that *Ascoidea hylecoeti*, and fungi associated with *X. lineatus* (*Monilia ferruginea*) were absent from the sites he studied. The association of *Ceratocystis piceae* with insects (*Tomicus piniperda* L.) was

reported earlier and a species of *Ceratocystis* was found in association with *Gnathotricus sulcatus* (Bakshi, 1950). The former worker believed that the fungus was probably *C. piceae*. Francke-Grosmann (1967) reviewed the ambrosial relationships between insects and blue-stain fungi. Tables 6 and 7 summarise such information. Other blue-stain fungi are associated with bark beetles (Table 7). However, these fungi are not considered ambrosia, but occur in the galleries during the beetles' greatest activity; in older galleries a complex of different fungi may be found. Some blue-stain fungi (e.g. *C. piceae* and *L. lundbergii*) may be found as free-living forms (Bakshi, 1950).

2.2.3. Economic importance of blue-stain fungi

Blue-stain fungi include a number of species of considerable economic and ecological interest. These fungi live on nutritive substances present in the wood-cells, especially in the medullary rays and other parenchymatous cells; they attack lignified cell walls only to a limited extent but in the ray cells they may cause considerable destruction. Blue-stain fungi attack standing trees when their moisture content is low and timber at different stages of storage before it is completely seasoned. Blue-stain fungi can cause considerable economic losses which are very difficult to control particularly those caused by fungi which attack newly felled timber in the forest simultaneously with infestations of bark beetles. Fungi and insects form firm associations which are characterised as true mutualistic symbiosis.

Francke-Grosmann (1963) reported that some blue-stain fungi parasitize weakened trees and cause death. Blue-stain fungi in conifers may facilitate the establishment of decay fungi such as Basidiomycotina *Heterobasidium annosum* Bref., *Stereum sanguinolentum* (Alb. and Schw. ex Fr.) and *Peniophora gigantea* (Fr.).

Table 6: *Ceratocystis* SPECIES ASSOCIATED WITH BARK BEETLES*

FUNGUS	BEETLE IN EUROPE
C. penicillata (Grosm.) C. Moreau	*Ips typographus* *Hylurgops palliatus* Gyll. *Hylastes cunicularius* Er. *Pityogenes chalcographus* L. *Pityogenes quardidens*
C. ips C. Moreau	*Ips* sexdentatus Boern *Ips sexdentatus*, *Orthotomicus proximus* Eichh.
C. brunneo-ciliata (M.-Kää.) Hunt	*Ips sexdentatus*
C. cana (Munch) C. Moreau	*Tomicus minor* Htg.
C. clavata (Math.) Hunt	*Ips acuminatus* Gyll. *Ips sexdentatus*
C. autographa Bakshi	*Dryocoetes autographus* Ratz. *Hylurgops palliatus*
C. galeiformis Bakshi	*H. palliatus*, *H. cunicularius* *Dryocoetes autographus*
C. polonica (Siem.) C. Moreau	*Ips typographus*
C. floccosa (Math.) Hunt	*Ips typographus*
C. albida (Math.-Kää.) Hunt	*Ips typographus*
C. minuta (Siem.) Hunt	*Ips typographus* *Tomicus minor* Htg.

(Contd...)

Table 6: *Ceratocystis* SPECIES ASSOCIATED WITH BARK BEETLES

FUNGUS	BEETLE IN NORTH AMERICA
C. ips	*Ips calligraphus* Germ. *Ips grandicollis* Eichh. *Ips pini* Say, *Ips avulsus* Eichh. *Ips oregoni* Eichh. *Ips plastographus* Le Conte *Ips lecontei* Sw. *Ips ponderosae* Sw., *Ips confusus* Lec. *Ips emarginatus* Lec. *Dendroctonus valens* Lec. *Dendroctonus monticolae* Hpk.
C. dryocoeti Kendrik & Molnar	*Dryocoetes confusus* Sw.
C. minor (Hedc.) Hunt	*Dendroctonus frontalis* Zimm. *D. brevicormis* Lec.
C. minor (=*C. pseudotsugae*) Rumb.	*D. pseudotsugae* Hopk.
C. piceaperda (Rumb.)	*D. piceaperda* Hopk. C. Moreau
C. montium (Rumb.) Hunt	*D. ponderosae* Hopk. *D. jeffreyi* Hopk., *emargnatus* Lec. *Ips confusus*
C. shrenkiana (Rumb.)	*Dendroctonus monticolae* *D. monticolae*, Hunt
C. huntii Rob. et Gvinch.	*D. valens* *D. monticolae*

* = Cited in: Francke-Grosmann (1967).

Table 7 : SPECIES OF *Hyphomycetes* FOUND AS AMBROSIA FUNGI WITH PHLOEOPHAGOUS BARK BEETLES *

FUNGUS	BARK BEETLES
Trichosporium symbioticum Wright	*Scolytus ventralis* Say
T. tingens Lagerberg and Melin	*Myelophilus minor* Htg.
T. tingens var. *macrosporum* Fr.-Gr.	*Ips acuminatus* Gyll.
Tuberculariella ips Leach	*Ips sexedentatus* Boern
	Ips pipni
	Ips grandicollis
	Ips sexdentatus

* = Cited in: Francke Grosmann (1967).

2.3. Ambrosia fungi in Britain

2.3.1. *Ceratocystis piceae*

Hunt (1956) noted that *Ceratocystis piceae* was first described in Germany on spruce and fir, but also is reported in England. *C. piceae* is commonly encountered on conifer logs and lumber and less frequently on hardwoods. However, it is not a serious cause of stain. In Minnesota *C. piceae* is also found on sporulating mats of *Ceratocystis fagacearum* (Gibbs, 1980). The former worker provided evidence that the rapid colonization of fresh wounds of red oak by *C. piceae* may play a vital role in limiting infection by *C. fagacearum*.

2.3.2. *Leptographium lundbergii*

The species has been established as an ambrosia for a long time and it was in fact believed that all ambrosia fungi from different parts of the world belonged to the genus *Leptographium* and could probably in many cases be referred to as *Leptographium lundbergii*. The genus was created and described in Sweden, and has been recorded in Australia and the United States and therefore, appears to have a wide geographical distribution. In Britain, the fungus was probably observed in 1922 but its independent position was not ascertained and the record by Bakshi (1950) was the first aunthentic one for Britain. *L. lundbergii* and two yeasts belonging to *Endomycopsis* were isolated from the tunnels of *Platypus subgranosus* (Platypodidae). The fungus was also isolated from pine by Bakshi (1950) and was found to be a strong blueing agent. The fungus grows readily on pine, spruce and larch. The hyphae penetrate the wood readily and are found abundantly in the pith rays as black lines filled with thick-walled, dark brown hyphae. They are also present in tracheids. Penetration between adjoining tracheids occurs frequently through bordered pits.

2.3.3. *Oedocephalum lineatum*

The fungus grows readily, producing a white mycelium. Aerial mycelium is at first scanty but soon numerous conidiophores appear and this gives the mat a powdery appearance, chalk-white in colour. The rate of growth is about 5cm in ten days. The fungus develops readily on all kinds of wood used for *C. piceae* and produces a white mycelium and abundant conidiophores.

2.3.4. *Ceratocystis ambrosia*

Cultural characteristics of *C. ambrosia* were described by Bakshi (1950).

2.4. Nutrition and growth of blue-stain fungi

2.4.1. Wood as a source of nutrients

Ceratocystis piceae grows more rapidly on sapwood than heartwood of balsam fir [*Abies balsamea* (L.) Miller]. and coremia were produced only on sapwood, and the fungus did not produce coremia on (2%) malt agar (Hubbes, Neumann and Willemot, 1976).

2.4.2. Growth of blue-stain fungi on agar

Mathiesen-Käärik (1960) reported the growth of a range of blueing fungi on different carbon sources, he also studied growth on various nitrogen sources, including calcium nitrate, ammonium sulphate, ammonium tartrate, asparagine, glutamine and alanine. *Trichosporium tingens* was unable to grow on these nitrogen sources in a purely synthetic medium. *Ceratocystis ips* was the only other fungus which could not utilize ammonium sulphate; apart from these two all the fungi grew on all the nitrogen compounds tested except nitrate. Of the 23 fungi tested only 9 utilize nitrate, most of them secondary blueing fungi.

The species of fungi showed total deficiency in thiamine, pyridoxine and synthetic biotin. Lilly and Barnett (1951) had earlier reported that most *Ceratocystis* species show deficiency for the three vitamins.

The influence of temperature on the growth of *Ceratocystis* spp was reported by Bakshi (1950) and Butin (1967). The morphology of *Ceratocystis* spp is highly dependent on the temperature of incubation, but growth is obtained at all temperatures from 4 to 30°C., with most rapid growth between 25 and 30°C. Higher temperatures are inhibitory for cell division and very poor growth is obtained over 31°C. (Höfsten, 1956).

Ceratocystis spp are best adapted to low pH, usually in the range 5.3 to

7.0 (Hofsten, 1956) or 4.0 to 6.0 (Puhalla and Bell, 1981). *C. piceae* was found to produce perithecia on only very acid media.

2.5. Objectives

It was found necessary that this study on the Scottish race of *Hylecoetus* should pay attention to the following: (1) life cycle and brood development; (2) relationships between emergence, flight and weather parameters; (3) host selection i.e. identification of the primary attractant for *Hylecoetus* in the field; (4) isolation and identification of the ambrosia fungus for *Hylecoetus*; and (5) nutritional and growth requirements of *Hylecoetus* and its ambrosia fungus.

CHAPTER 3

CHAPTER 3

MATERIALS AND METHODS

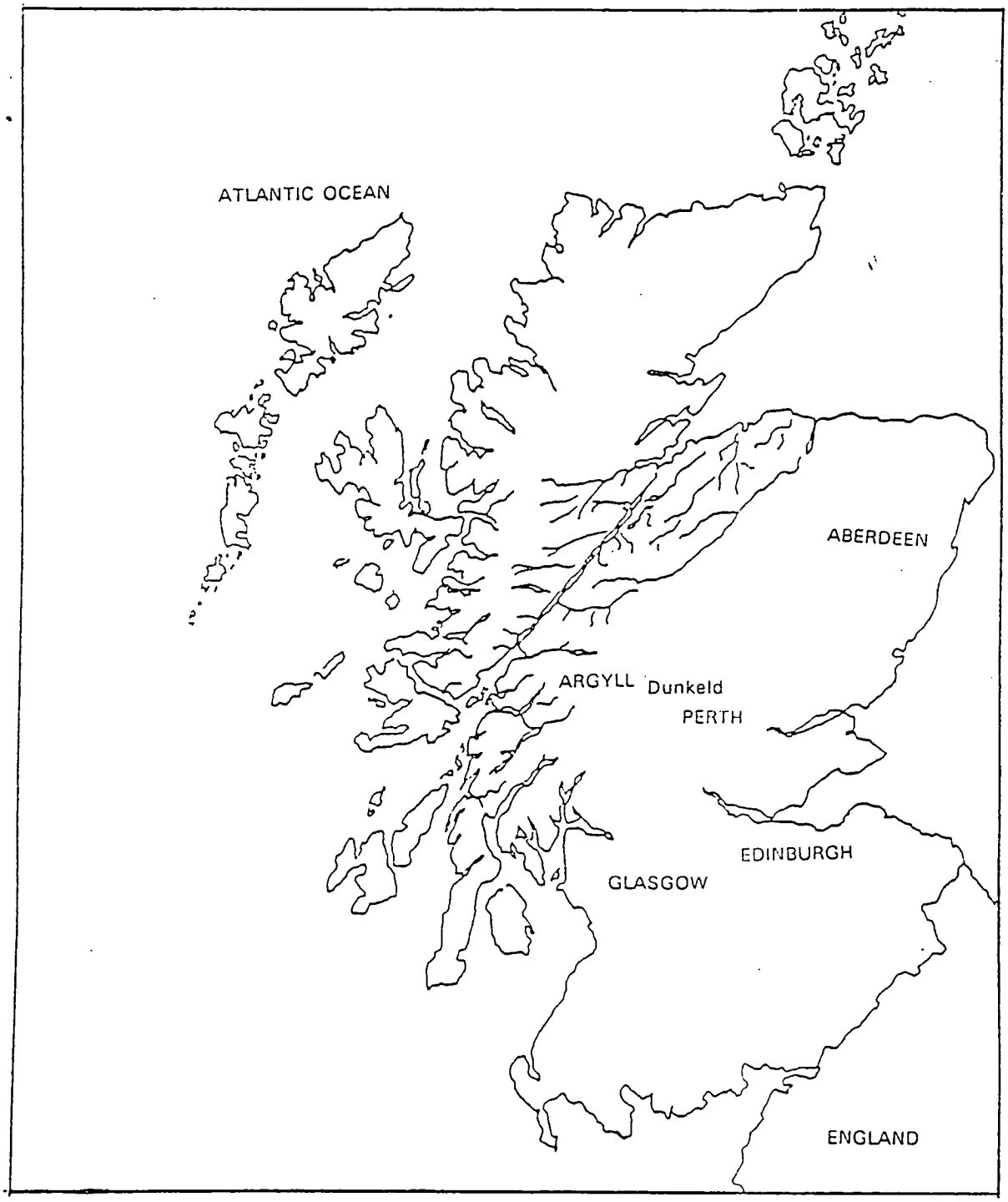
3.1. Description of the study area.

Field work was conducted in Perthshire (Map 1) at Dunkeld in both Atholl (National Grid Reference: NO 0245) and Craigvinean (National Grid Reference: NN 4698) forests (Map 2). The major sites where field work was carried out in 1983-1986 are shown on Maps 2 and 3 , and further information on the sites is given in Table 8. Sites 1-30 were on Atholl Estate, and sites 31-34 were in Craigvinean forest. Laboratory work was carried out in the Department of Forestry and Natural Resources, University of Edinburgh.

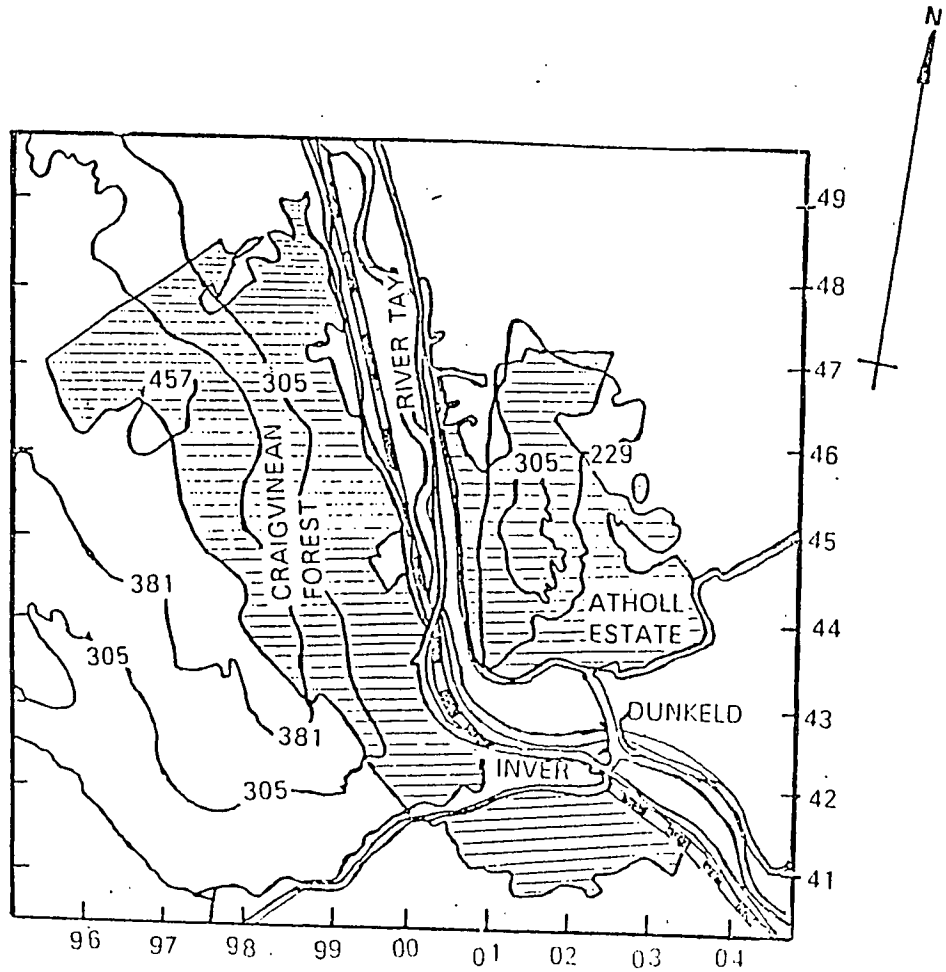
3.2. Emergence and flight of *Hylecoetus*

3.2.1. Emergence of adults

A rearing cage was constructed from a plywood box (60x60x60cm) with a sliding lid. Each side of the box was connected by a 15cm. long, clear plastic tube to a 30x30x30cm insect diet cage (Fig: 7). This apparatus was kept at Inver, Dunkeld in April 1984 (Site 30). The four diet cages were facing the cardinal points north, east, south and west. Brood material consisting of *Hylecoetus* was cut from stumps at site 20, and placed in the plywood box, prior to the time of the main flight. Emerging *H. dermestoides* were collected from the diet cages daily up to the end of June. *Hylecoetus* recovered from the cages were sexed, using methods described by Fergusson (1920) and Thomsen *et al*. (1949). Temperature and rainfall were recorded at site 29.



Map 1: Map of Scotland; Scale: 1:2,500,000



Map 2: Map of Dunkeld showing the Atholl and Craigvinean forests and Inver.

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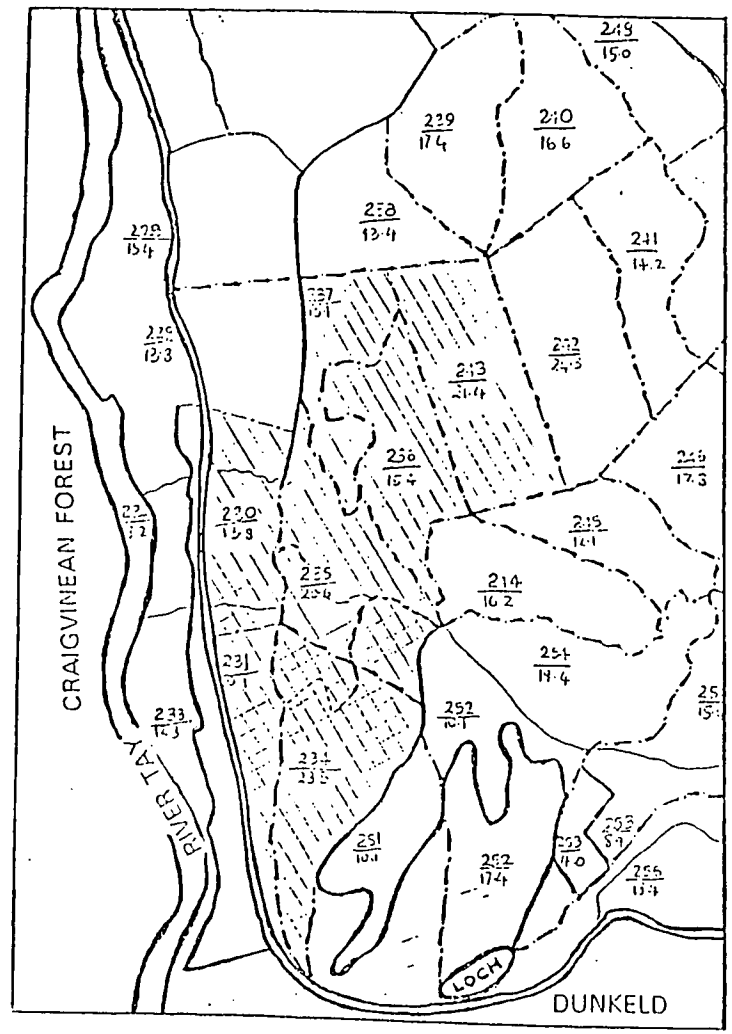


Map 3: Map of Atholl estate showing the compartments (sites) where work was carried out in 1983-1986.

237 / 10·1 COMPARTMENT SAMPLED

Table 8: SUMMARY OF INFORMATION ON SITES INDICATED ON MAP 2.

SITE NO.	COMPT	SPECIES	PLANTED	FELLED	ACTIVITY
1	237	larch	1951	Oct.1983	Larch logs used as bait for window barrier trap No.1
2	230	larch	1926	March.84	Single tree felled for bait logs for window barrier trap No.2
3	230	larch	1926	March.84	Single tree felled for bait logs for window barrier trap No.3
4	230	larch	1926	April.84	Single tree felled for window barrier trap No.4
5	230	larch	1926	Oct82-May83	Larch logs used as bait for cross vane trap No.1
6	230	larch	1926	Oct82-May83	Larch logs used as bait for cross vane trap No.2
7	230	larch	1926	Oct82-May83	Larch logs used as bait for cross vane trap No.3
8	235	larch	1926	Oct.,83	Cleared site, stumps used as bait for 6 cross vane trap(Nos A-F)
9	235	larch	1926	-	Standing dead trap trees Nos 1-6, and 11-12
10	230	larch	1926	-	Standing dead trap trees trap trees Nos 7-9
11	234	larch	1926	-	Standing dead trap tree No.10
12	235	larch spruce Scotspine	1926	-	Multiple funnel traps set up in larch area
13	237	larch	1951	-	Window barrier trap baited with ethanol
14	236	larch	1926	-	Replicated trapping experiment using ethanol as bait
15	235	larch spruce Scots pine	1926	-	Block A of randomised block design set up in larch area
16	236	larch	1926	-	Block B of randomised trapping experiment
17	236	larch	1926	-	Block C of randomised trapping experiment
18	236	larch	1926	-	Block D of randomised trapping experiment
19	230a	larch	1926	Oct.82-May.83	Cleared area, collection of *Hylecoetus* specimens from stumps
20	236a	larch	1926	Feb.&Sept.83	Cleared area, collection of *Hylecoetus* specimens
21	243a	larch	1926	Oct.82/May.83	Cleared area, collection of *Hylecoetus* specimens
22	234	larch	1926	Jan-Feb.82	Cleared area., collection of *Hylecoetus* specimens
23	234	larch spruce Scots pine	1926	Sept-Oct.,84	Selectively thinned, collection of *Hylecoetus* specimens
24	235	larch spruce Scots pine	1926	Sept-Oct.,84	Cleared area, collection of *Hylecoetus* specimens
25	235	larch spruce Scots pine	1926	Sept.-Oct.,84	Cleared area, collection of *Hylecoetus* specimens
26	235	larch spruce Scots pine	1926	Sept-Oct.84	Spruce clear felled, larch selectively thinned, Stumps of both species infested with *Hylecoetus*
27	236	larch	1926	Sept-Oct.84	Cleared area, collection of *Hylecoetus* specimens
28	230a	larch	1926	Oct.82/May.83	Cleared area, weather station
29	-	-	-	-	Weather station at Tayside Forestry Commission Offices,Inver.
30	-	-	-	-	Farm shed with emergence cages
31	-	spruce	-	-	3 cross vane traps baited with ethanol
32	-	larch	-	-	Selectively thinned, larch logs used as bait for window barrier trap
33	14	Spruce	1920	Feb.,83	Cleared area, collection of *Hylecoetus* specimens
34	7a	Spruce	1939	Oct-Nov.,83	Cleared area, collection of *Hylecoetus* specimens

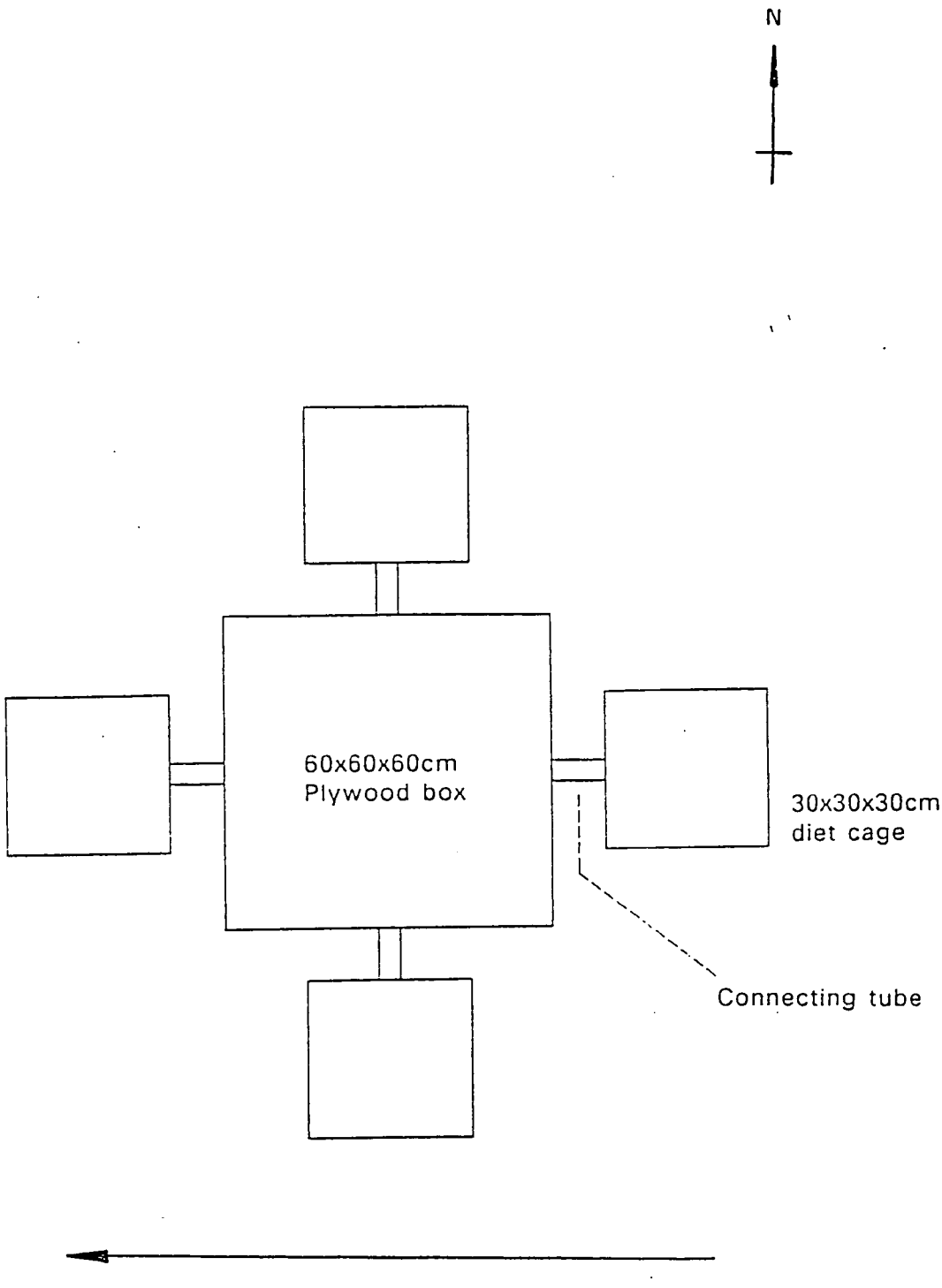


Fig: 7. Arrangement of emergence boxes containing pieces of larch wood infested with *H. dermestoides* brood. The boxes were kept in the field at Inver, Dunkeld from 2 May to 30 June, 1984.

3.2.2. Flight

3.2.2.1. Trap design

Little information on the trapping of *H. dermestoides* is available. A flight trap constructed with sheets of muslin heavily covered with grease, and a Rothamsted-type mercury vapour light trap (Williams, 1948) were used in an unsuccessful attempt to catch *Hylecoetus* and *Xyloterus* beetles in Argyllshire (Map 1) by Bletchly and White (1962). Of the two species *H. dermestoides* and *H. flabellicornis*, only the latter was found in window flight traps in Finland by Löyttyniemi and Uusvaara (1977) (Table 1). Although both Thomsen *et al* . (1949) and Kurir (1972) worked in Denmark and Austria respectively on *H. dermestoides* they did not give information on the methods used to sample the population. Trap designs suitable for Coleoptera have been tried by different workers as follows: (1) the window flight trap (Chapman and Kinghorn, 1955, 1958; Bakke, 1968 and Bakke, Austara and Pettersen, 1977); (2) cross vane trap (Bakke, 1975); and (3) multiple funnel trap (Lindgren, 1983; Lindgren, Borden, Chong, Friskie and Orr, 1983).

3.2.2.2. Collection of flight data.

Flying *H. dermestoides* were sampled on a daily basis from 18 April to 30 June, 1984 and 8 May to 5 July, 1985. *Hylecoetus* were sampled on an hourly basis on a few days only in 1984. *Hylecoetus* were collected from 10.00 to 20.00HRS on days with favourable weather between 13 May and 30 June. *Hylecoetus* were caught by window flight traps (Fig: 8), cross vane traps (Fig: 9), multiple funnel traps (Fig: 10) and a modification of the window trap (Fig: 11). Larch logs, stumps, standing dying or dead trees, and semiochemical attractants were used as baits. A summary of information on trap design, baits and trapping dates for 1984 is given in Table 33. Little information and no flight data at all were collected in 1985 and 1986 due to poor weather.

Fig: 8 The window barrier flight trap used for studies on *H. dermestoides* at Dunkeld in 1984.

Fig:9 The cross vane flight trap used for studies on *H. dermestoides* at Dunkeld in 1984.

Fig:10 The multiple funnel flight trap used for studies on *H. dermestoides* at Dunkeld in 1984.

Fig: 11 The modified window barrier flight trap used for studies on *H. dermestoides* at Dunkeld in 1985.

In 1984 the number of traps operated varied between days (Table 33). Therefore, a "flight index" obtained by dividing the day's total catch by the number of traps was used to express daily *Hylecoetus* activity. The flight index was used to establish the seasonal flight pattern for 1984, while total catch was used in 1985. The hourly catch was used to establish the diurnal flight pattern in 1984. The diurnal flight pattern in 1985 was not studied due to poor weather. Weather data: maximum and minimum air temperature, minimum grass temperature, soil temperature at the 10 cm depth, rainfall, relative wind speed, relative humidity and sunshine hours were obtained for Ardtalnaig (NN 7139, about 20.5 miles west of Dunkeld) from the Meteorological Office, Forestry Commission, Corstorphine, Edinburgh. Hourly weather data: dry and wet bulb temperatures using a whirling hygrometer; relative wind speed using a cup anemometer were recorded at site 28 in 1984. Hourly relative humidity data were derived from wet and dry bulb readings and was assisted by Dr. J. Grace.

3.3. Host selection – primary attraction

3.3.1. Response of *H. dermestoides* to larch logs

At the end of March, 1984 four groups of larch logs felled at different times of the year were available on the Atholl Estate (Table 9). Groups of logs were located at least 100m apart. A window flight trap was set up above the logs as described by Bakke (1968). *Hylecoetus* were collected from the traps from 18 April to 5 June, 1984 on a daily basis.

3.3.2. Response of *H. dermestoides* to larch stumps

During the fourth week of April, 1984 *Hylecoetus* were observed landing on larch stumps at site 8. A cross vane trap was fixed above each of 6 larch stumps which were attractive to *Hylecoetus*. Plastic trays Invictor Decor, IP4810 ® – 298 mm 11 3/4" were placed above the stumps to catch the falling

Hylecoetus (Fig: 12). Trays contained water and a little detergent used as a wetting agent. Average distance between traps was 12m. *Hylecoetus* were collected from the traps from 28 April to 5 June, 1984 on a daily basis.

Table 9: SUMMARY OF INFORMATION ON TRAP LOGS FOR WINDOW BARRIER TRAPS Nos 1-4 AT SITE Nos 1-4 (Table 16)

SITE NO.	TRAP NO.	NO. OF LOGS	MEAN LENGTH (m)	MEAN MID DIAMETER (cm)	FELLING DATE
1	1	7	1.90	18.0	Nov., 1983
2	2	9	1.72	22.0	March, 1984
3	3	8	1.90	21.5	March, 1984
4	4	8	1.70	17.8	April, 1984

3.3.3. Response of *H. dermestoides* to standing dead larch trees

In May, 1984 *Hylecoetus* were observed landing on standing dying or dead larch trees. Twelve of these trees were chosen as trap trees (sites 9-11). Distance between trees ranged from 7 to 40m. The trees were inspected from 13 May to 30 June on an hourly basis between 10.00 and 20.00HRS. *Hylecoetus* which landed on trees rarely flew away again and were therefore, picked by hand.

3.3.4. Response of *H. dermestoides* to semiochemicals

3.3.4.1. Response to ethanol

A window flight trap was erected inside a larch stand (site 13). Ethanol (70%) held in two plastic vials (ca.63.4mm height, 8.4mm i.d., 1.4mm thick) and released by diffusion through two holes (aperture diameter, 1.5mm) made through the caps, was used as bait. Ethanol dispensers were fixed by sticky tape to the trap so that the caps were just above the top edge of the window.

Fig: 12 The cross vane flight trap placed on larch stump used for studies on *H. dermestoides* at Dunkeld in 1984.

Hylecoetus were collected from the trap from 29 May to 30 June, 1984.

A further nine traps were set up to give 3 replications i.e. 3 levels of alcohol as follows: 2 vials, 1 vial per trap and a blank control. *Hylecoetus* were collected from the traps from 9 June to 30 June, 1984.

3.3.4.2. Response to alcohols, monoterpenes and a pheromone

A range of the monoterpenes found in European larch by Stairs (1968), alcohols produced by conifers (Moeck, 1970; Cade et al ., 1970) and lineatin, a pheromone for *Xyloterus lineatus* were field-tested in modified window flight traps (Fig: 11). These chemicals (Table 10) were used in a Randomized Block Design, with 4 blocks. Each block was a row set up with a minimum distance of 25m between traps (Stoakley, Bakke, Renwick and Vité, 1978; Conn, Borden, Scott, Friskie, Pierce and Oehlschlager, 1983). Blocks were at least 100m apart (sites 15–18). The blocks were selected so that they were homogenous with regard to trap exposure and proximity to infested stumps. Each treatment had

Table 10: SOURCE, PURITY, SOLVENT AND RELEASE DEVICE FOR CANDIDATE SEMIOCHEMICAL ATTRACTANTS FOR *Hylecoetus dermestoides*

COMPOUND	SOURCE	CHEMICAL PURITY	SOLVENT	RELEASE DEVICE
α–pinene	Fluka	>97%	–	Glass vial
β–pinene	Fluka	80–90%	–	Glass vial
Camphene	Fluka	~99%	Eth/Chlo [1]	Glass vial
Limonene	Fluka	>98%	–	Glass vial
Myrcene	Koch Light	–	–	Glass vial
Ethanol	–	95%	–	Glass vial
Methanol	–	–	–	Glass vial
Chloroform	–	–	–	Glass vial
Lineatin	Alice Holt [2]	–	–	Fibre

1. Eth/Chlo [1] = ethanol plus chloroform

2. Alice Holt [2] = Alice Holt Lodge, Forestry Commission, Surrey, England.

one replicate in each block. Traps were placed on the forest floor. Alcohols and monoterpenes were dispensed from glass vials (ca. 75mm height, 25mm i.d.). The open ends of the vials were sealed with brown packing tape. A small hole (2-4mm diameter) was made through the tape cover to release the attractant. Lineatin was held in fibre dispensers in polythene covers-a type of Hercon dispenser as described by Quisumbing and Kydonieus (1982). *H. dermestoides* were collected from the traps from 14 May to 4 July, 1985.

3.4. Life cycle and brood development

3.4.1. Pairing

Pairing by *H. dermestoides* was observed under natural conditions on the Atholl Estate in 1984. Paired *Hylecoetus* were looked for on stumps, standing dead trees, in flight traps and emergence cages. Pairing was observed from 18 April to 30 June. The time of day at which each pair was observed was recorded. All pairs were hand picked and stored in 1oz. glass phials for oviposition.

3.4.2. Oviposition

As many of the adult female *Hylecoetus* as possible obtained from traps, stumps, standing dead trees and emergence cages were kept alive in 1oz. phials for oviposition. A piece of filter paper was enclosed inside the phial for the female *Hylecoetus* to rest on, and the piece of paper provided physical support for the extruding ovipositor. The ovipositor rubs against rough surfaces to facilitate egg laying. Batches of eggs deposited and number of eggs per batch were recorded. Oviposition was also observed for adult *Hylecoetus* which had emerged from pupae on artificial media in the laboratory.

3.4.3. Incubation and hatching

Incubation period and hatching were observed on eggs deposited by adult *Hylecoetus* emerging from pupae on artificial media. Agar media and larvae were kept in the dark in an incubator at 20±5°C.

3.4.4. Survival and development under controlled conditions

In addition to rearing larvae on artificial media, larvae were reared under controlled conditions by cutting brood material from infested stumps and reducing the wood to slabs to fit into plastic trays (33x20x6.5cm). The wood rested on 4-5, 10-15mm high vinyl plastic supports cut from a pipe (ca. 3.8cm i.d.) (Fig: 13). 250ml of a saturated salt solution (distilled water and sodium nitrate, $NaNO_3$) was poured into each tray to make the relative humidity at approximately 75 percent (O'Brien, 1948). When salts were not available only distilled water was used which produced relative humidity of approximately 100 percent (O'Brien, 1948). Nystatin (Mycostatin-Squibb) was used to control microbial growth in the trays and was added to give an inhibitory concentration of 1 unit/ml. A second tray was used as cover. Trays were kept in a growth cabinet at 20°C. A hygrothermograph was kept inside the growth cabinet to monitor temperature and relative humidity continuously. The salt solution or the distilled water was replenished as often as possible. The method does not allow direct observation of developing *Hylecoetus* like the bark sandwiches used by Bedard (1933) to rear *Dendroctonus pseudotsugae* Hopk. (Scolytidae) and Balogun (1970) to rear *Ips cembrae* Heer (Scolytidae). However, *Hylecoetus* bred successfully up to the adult stage. Since the bark beetles develop between the bark and sapwood, they were successfully reared by clamping infested pieces of bark (5x7 in) between two plates of glass (8x9 in). Moist cotton was packed around the bark. The device was wrapped in a black cloth. The beetles were examined by direct observation.

Fig 13: Rearing *H. dermestoides* infested piece of wood of larch with heaps of bore-meal produced by larvae. Covered trays were kept at 20±5°C., and relative humidity at 75-100%.

3.4.5. Larval instars and pupation

Hylecoetus larvae from stumps, trays and agar plates were fixed on petroleum jelly and mounted on a travelling microscope for examination of morphological characters, distinguishing different larval instars as described by Thomsen (1950). Headcapsule widths, body size (length) and length of "tail" were measured to the nearest 0.02mm (at 10x). Headcapsule width was measured as described by Bedard (1933). Dyar's rule (Dyar, 1890) and frequency distributions based on headcapsule widths were used to determine the number of larval instars as described by Zethner-Møller and Rudinsky (1967); Ngoan, Wilkinson, Short, Moses and Mangold (1976) and Hara and Beardsley, Jr. (1979) etc. Counts of moults by larvae of *Hylecoetus* on agar media were recorded.

Pupation was observed on insect material on agar media.

3.5. Ambrosia beetle-fungus symbiosis

3.5.1. Isolation and identification of ambrosia fungi

Stumps containing galleries of *H. dermestoides* were identified by the obvious presence of white, coarse bore-dust on the bark surface. The presence of active brood was determined by cutting into galleries underlying detected heaps of bore-dust. Stumps containing active galleries were cut with a power saw. These pieces of wood were brought to the laboratory for dissection and possible microbial isolations. The ambrosia fungi for *Hylecoetus* were isolated from various sources as follows: (1) larval and pupal tunnels (Batra and Francke-Grosmann, 1961); (2) egg shells (Kurir, 1972); (3) larvae; (4) adult *Hylecoetus* Batra, 1963a, 1967); and (5) bore-meal (Peplinski and Merill, 1974).

3.5.1.1. Tunnels

Wood slivers (Bakshi, 1950; Batra, 1967) from actively tended tunnels were surface sterilized by dipping into 0.1% silver nitrate solution; 0.1% sodium chloride solution and sterile distilled water (twice) for about one minute

respectively (Hayes, 1965). Slivers were aseptically plated onto disposable Petri-dishes containing 15-20ml of agar media, malt extract agar (MEA); malt extract agar plus yeast extract (YEME); Czapek dox agar (modified) (CzDA); potato dextrose agar (PDA); corn meal agar (CMA) and plain agar. Scrapings off tunnel walls were also plated on agar.

3.5.1.2. Larva

Larvae collected from stumps were surface sterilized (Hayes, 1965) and placed aseptically onto agar media. Fungi were isolated from traces of the larval excrement on agar.

Alimentary canals were dissected aseptically from larvae, and their contents were plated on agar (Bakshi, 1950).

3.5.1.3. Adult

Ovipositors and associated mycangia of female *Hylecoetus* were severed aseptically from the body, crushed and plated on agar media. Isolations were also made by allowing adult *Hylecoetus* to walk on agar plates.

3.5.1.4. Eggs

Ambrosia fungi were isolated from eggs laid by female *Hylecoetus* on agar in the laboratory.

3.5.1.5. Bore-meal

Fresh bore-meal was plated on agar media.

3.5.1.6. Identification of fungi

Cultures were checked as often as possible for microbial growth. Identification of cultures was assisted by Dr. A.J. Hayes.

3.5.2. Seasonal occurrence of ambrosia fungi in *Hylecoetus* larvae

The objective was to study the seasonal association of the ambrosia fungi and *Hylecoetus* larvae. Larvae representing various instars were collected from stumps or tray chambers in the growth cabinet. The fungi were isolated from larval faecal traces on agar plates and sections of the alimentary canal from 28 September, 1985 to 14 May, 1986.

3.5.3. Wood as a source of nutrients

3.5.3.1. Preparation of bark, wood and bore-meal samples

Billets about 20cm long and 20cm diameter were cut from healthy larch trees on the Atholl Estate. Billets were cleaned by removing the outer bark before separating the inner bark from the wood core. Inner bark was cut into strips and air dried. Sapwood was separated from heartwood. The wood was cut into pieces about 1x1x1cm then air dried. Dried bark and wood was ground in a No.8 and Retsch-Type 17-140 (from Glen Creston) laboratory mills. Fresh bore-meal was collected from the surfaces of infested stumps.

3.5.3.2. Preparation of sugar and amino acid extracts

Sugar and amino acid extracts were prepared using a technique described by Shiroya, Slankis, Krotkov and Nelson (1962) and adapted by Nelson (1964) and Balogun (1965). Finely ground bark (10g) and sapwood (10g) and bore-meal (2.6g) was extracted in 100ml distilled water at 37°C. for 3h. Material was filtered under vacuum. The residue was re-extracted at 37°C. for 3h. Both extracts were combined and dried *in vacuo* at 60°C. using a Rota Vapor. The dried extract was taken up in 20ml distilled water. It was separated by means of resins IR-120(H^+) and IRA-400(Cl^-) into sugar, amino and organic acid fractions (Shiroya *et al* ., 1962). Each amino acid and sugar fraction was dried at 60°C. in a Rota Vapor. The dried extract was re-dissolved in 10 ml distilled water and resolved by two-directional thin-layer chromatography (TLC).

Amino acid samples from bark, wood and bore-meal were further resolved quantitatively using an Automatic Amino Acid Analyzer. The wood (0.5g) and bark residue (0.5g) was extracted in 6*N* HCl in a Soxhlet apparatus for 10h to obtain protein bound amino acids. The extract was filtered, dried, re-dissolved in 20ml distilled water and then separated on resin columns. The amino acid extract was dried then re-dissolved in 10ml water and resolved qualitatively and quantitatively.

3.5.3.3. Thin-layer chromatography (TLC)

Determinations of the qualitative composition of sugars and amino acids extracted from larch bark and wood were carried out using TLC procedures. Only procedures which gave the best separations of amino acids and sugars are described below. TLC of amino acids was carried out as follows:

1. Cellulose 144, 250μ thick layers were used.

2. Tank No.1 was completely lined with Whatman No.2 filter paper, and then solvent No. 1 (n-butanol-{80ml}-glacial acetic acid {20ml}-water{20ml}) was poured into the tank. The tank was thoroughly shaken to ensure that the filter paper adhered to the walls of the tank. The tank was closed tightly, and then it was placed in a fume cabinet for tank saturation for 60 minutes.

3. The boundary line was marked only at the edges of the plates, and was 10cm above the spotting points, on each of the 20x20cm plates. Standard amino acids were dissolved in 0.1 N HCl, and each spot contained 0.5μg/0.5μl, of each of the amino acids in the sample. 1.5 to 2.0μl of each standard and 10μl of each of the unknown sample was spotted. The spots were dried using a current of air from a hair dryer.

4. Two-spotted plates were placed into the tank in a `V`, and then the tank was closed and placed in the fume cabinet. Chromatograms were run in the first phase for about 120 minutes.

5. While running the chromatograms in the first direction, solvent No.2 [phenol(75g)-water(25g)(NaCN, 20mg per 100g mixture), was prepared and the tank saturation was carried out as described above. The second phase, with a solvent front of 10 cm, was achieved after 190 minutes.

6. After the first direction was completed, the solvent front

was immediately marked on each plate. The plates were then dried on a rack in the fume cabinet for 15 to 20 minutes. The plates were further spotted, before they were run in the second direction in tank No. 2. After the second run, the solvent front was marked on each plate, and then the plates were dried as described above. The dried plates were sprayed with ninhydrin reagent No. 108 [ninhydrin (0.3g), n-butanol (100ml) and glacial acetic acid (3ml)] (Stahl, 1965).

7. The sprayed plates were heated at 110°C. for 15 to 20 minutes. The developed spots on the chromatograms, were outlined with a sharp pencil. Before storage, the plates were sprayed with a colour stabilizer reagent No. 108 [saturated aqueous copper nitrate solution (1ml), 10% nitric acid 0.2ml and 95% ethanol (100ml)]. The plates were left on a rack in a fume cabinet with a beaker of ammonia solution.

Two-dimensional TLC of sugars extracted from bore-meal and sapwood was carried out as follows: a total of 2.5g of *Hylecoetus* bore-meal was collected from the surfaces of 7 larch stumps on the Atholl estate, on 4 July, 1985. The bore-meal was collected with a spatula and directly stored in 1 oz. phials of 70 percent ethanol. Sugars in wood and bore-meal were extracted using a total of 150ml cold 70 percent ethanol using a method which followed procedures decsribed by Shiroya *et al*. (1962) and Nelson (1964). The following were the main features of the methodology used:

1. Ordinary silicagel H 250μ thick layers prepared with 0.02 M sodium acetate were used in both directions. V-shaped wedge strips were used for mixtures of sugars, and ordinary spots were used for solutions of single sugars.

2. N-butanol-glacial acetic acid-diethylether-water (9:6:3:1) was used in the first dimension, and gave (1) good spot separations, with good colour development, (2) no splitting of individual sugar spots. The first run was completed after 80 minutes.

3. N-butanol-acetone-water (4:5:1) was used in the second dimension. This solvent gave fair spot separation and splitting of individual sugars did not occur. The second phase was completed after 40 minutes.

4. Plates were conditioned by heating at 120°C. for about 30 minutes and stored over NaOH before use. 10 μl of each of the 5% standard sugar solutions, giving 0.5g/sugar, and 10μl of each of the wood and bore-meal extracts were

spotted onto the plates.

5. After the second phase chromatograms were sprayed with freshly prepared reagent No. 9(c) or No. 15.1 (a) (anisaldehyde {5ml}, ethanol [95%] {90ml}, concentrated sulphuric acid {5ml}, and glacial acetic acid {10ml}) Stahl (1965) and Randerath (1968) respectively.

6. After spraying, plates were heated at 120°C. for about 10 to 15 minutes. The pink background which developed on the plates, was brightened by treatment with water vapour over a steam bath.

3.5.3.4. Quantitative amino acid analysis

Total concentrations of amino acids in bark, wood and bore-meal were determined. Preliminary paper electrophoresis at pH 2.1 indicated that samples from resin columns [IR-120(H^+)] still contained some lignin, and also gave an estimate of the volume of sample that would be required for a quantitative analysis.

Amino acids and lignin were separated by the method described by Colowick and Kaplan (1972): sulphonated polystyrene beads (H^+ form) were added to 5ml of the amino acid extract until the pH of the supernatant after shaking was 2.5-3.0 (indicator paper). The mixture was shaken, the supernatant removed, and the beads were washed twice with 2 (resin) volumes of water. The adsorbed amino acids were then eluted with three 2-volume portions of 5 *M* ammonia solution and the combined eluates were evaporated to dryness in a vacuum desiccator.

The sample was re-dissolved in 100μl 0.1 *M* ammonia solution and 10 ul of this was run on High Voltage Electrophoresis (H.V.E.P.) at pH 2.1 to identify the amino acids present and to confirm that the sample was now clean of lignin. The remaining 9/10 of the sample was re-dried, then dissolved in 150μl of citrate buffer pH 2.2 containing internal standard (not leucine) and the amino acids separated by ion-exchange chromatography on a Beckman Amino Acid Analyser (Model 120c).

The analytical procedure involves the addition of ninhydrin and the colorimetric determination of the acids at each of three wavelengths.

Comparative amounts (in micro-moles) of each amino acid in the sample can be calculated - see Amino Acid Analyser Chart and calculation sheet (Tables 46-49).

3.5.4. Growth studies of ambrosia fungi

The effects of various agar media, larch bark, sapwood and heartwood (ground as described above), individual carbon and nitrogen sources, temperature and pH, on the ambrosia fungi for *Hylecoetus* were studied.

3.5.4.1. Effects of agar and larch sapwood meal

Malt extract agar (oxoid) (5g/l) with (3g/l) Bacto yeast extract; potato dextrose agar (oxoid) (39g/l); corn meal agar (oxoid) (17g/l); Czapek dox agar (modified) (oxoid) (45g/l); nutrient agar (oxoid) (28g/l) and larch sapwood (30g/l) plus agar (12g/l) were sterilized by autoclaving at 120°C. for 15 minutes, then poured in Petri-dishes. Ten replicates for each treatment were used. Petri-dishes were inoculated uniformly by placing a 3mmx3mm inoculum cut from 12 day-old cultures, in the centre of the Petri-dish, and were incubated at 20°C. The diameters of the colonies were measured at the end of 7, 10, 13, 16 and 19 days. The means of two readings taken at right angles to each other were recorded (Hayes and Manap, 1975).

3.5.4.2. Effects of sapwood and heartwood

Finely ground sapwood (30g/l) plus agar (12g/l) and heartwood (30g/l) plus agar (12g/l) were prepared and inoculated. Thirteen cultures were used for each treatment. Measurements of the colony diameters were carried out after 5, 9, 13, 15, 17 and 19 days.

3.5.4.3. Effects of bark and sapwood

Pairs of bark and sapwood samples were collected from 5 healthy larch trees from Atholl Estate, and prepared as described above. Five replicates for each treatment were used. Colony diameters were measured after 3 days.

3.5.4.4. Effects of sapwood and sapwood extracts of sugars, amino and organic acids

Sapwood extracts were prepared using a modification of methods described by Shiroya *et al*. (1962) and Balogun (1965). Finely ground sapwood (20g) was extracted in 200ml cold 70% ethanol for 2h. The extract was filtered under vacuum, and the residue was re-extracted using cold 70% ethanol. The ethanol soluble portions were combined and dried *in vacuo* using a Rota Vapor at 60°C., and re-dissolved in 80ml water (SAO). Twenty ml of the extract was filter sterilized and added to 80ml autoclaved agar to give 15g/l agar in the medium, and dispensed in Petri-dishes (Treatment 7). Remaining (SAO) was separated into sugars combined with organic acids, and amino acid fractions, by placing 20ml portions on the resin column IR-120 (H^+). Eluate from the resin column contained the bulk of the sugars and organic acids (SO). Adsorbed amino acids were eluted with *N*-ammonium hydroxide per column, then the column was washed with 5ml water, and eluates were combined (AA). 20ml of (SO) was filter sterilized, added to agar and dispensed in Petri-dishes (Treatment 6). Sample (AA) was dried in a Rota Vapor and re-dissolved in 40ml water (AA1). 20ml of (AA1) was added to agar and poured in Petri-dishes (Treatment 1). The remainder of (SO) was placed in a resin column containing IRA-400 (Cl-). The eluate from this column contained the sugars (S). 20ml of (S) was added to agar (Treatment 2). Organic acids adsorbed on column IRA-400 (Cl^-) were eluted with a total of 30ml 5*N* Formic acid and 10ml water used to wash the column. The combined eluate was dried in a Rota Vapor and redissolved in 40ml water (OA). 20ml of (OA) was added to agar (Treatment 3).

A mixture of 20ml each of (AA1) and (S) were added to 160ml agar to give 15g/l agar in the medium (Treatment 4), and 20ml each of (AA1) and (OA) were combined and added to 160ml agar (Treatment 5). Sapwood meal was prepared as described above and was used as (Treatment 8). After each separation resin columns were regenerated using 5% w/v HCl (British Drug House, 1981). Cultures were inoculated and incubated as described above. Seven replicates for each treatment were used. Colony diameters were measured after 4, 6, 10, 13, 15 and 18 days.

3.5.4.5. Effects of temperature on fungi growth

Larch sapwood/agar (LSW) and Czapek dox agar (modified) (CzDA) were used. After inoculation the Petri-dishes were placed at 5, 10, 15, 20, 25, 30, 35 and 40°C. Four replicates for each treatment were used. Colony diameters were measured after 4, 6, 9, 12, 15, 18 and 22 days.

3.5.4.6. pH and fungal growth

Ambrosia fungi were grown on PDA adjusted to 17 different pH values as follows: 2.2, 2.6, 3.0, 3.4, 3.8, 4.0, 4.6, 5.4, 5.6, 5.8, 6.0, 6.4, 6.8, 7.4, 7.6, 7.8, and 8.0, using 0.1 *M* citric acid and 0.2 *M* Na_2HPO_4 buffer. Inoculated Petri-dishes were incubated at 20°C. 5 replicates for each treatment were used. Colony diameters were measured after 3, 7, 9, 11 and 19 days.

3.5.4.7. Carbon nutrition of ambrosia fungi

The influence of four sugars found in larch bark and wood, glucose (G), raffinose (R), fructose (F) and sucrose (S) on the growth of ambrosia fungi was examined as follows: combinations of one, two, three and four sugars were used. Each sugar was prepared in solution, autoclaved separately and then added to the basic medium at 5g/litre. Four replications for each treatment were used. Media were adjusted to pH 3.8 before pouring them into Petri-dishes. Cultures were grown at 20°C. Colony diameters were measured

after 5, 9, 13 and 20 days.

Basic medium

1. NH_4-tartrate	0.1g
2. KH_2PO_4	1.0g
3. $MgSO_4.7H_2O$	0.5g
4. Agar	15.0g
5. Distilled water	1 litre

The influence of 6 groups of carbon sources was examined as follows:

1. *Aldopentoses*: xylose, arabinose
2. *Aldohexoses*: glucose, mannose, galactose
3. *Ketohexoses*: fructose
4. *Disaccharides*: cellobiose, lactose, maltose, sucrose
5. *Trisaccharides*: raffinose, melezitose
6. *Polysaccharides*: cellulose, starch

Each carbon source was prepared in solution and was autoclaved separately then added to the basic medium, at 10g/l. Four replicates for each treatment were used. Media were adjusted to pH 3.8 before pouring them into Petri-dishes. Cultures were incubated at 20°C. Colony diameters were measured after 5, 9, 13 and 20 days. An experiment with sucrose concentrations 0.1, 0.2, 0.5, 1.0, 1.5, 2.5, 5.0, 10.0% and a control was carried out. Preparation of media, adjustment of pH, inoculation and incubation were carried out as described above. Colony diameters were measured after 5, 10, 15 and 20 days.

3.5.4.8. Inorganic nitrogen nutrition

The following inorganic nitrogen sources were added individually to the basic medium.

1. *Nitrate nitrogen*: $CaNO_3$, $NaNO_3$, KNO_3
2. *Ammonium nitrogen*: ammonium tartrate, NH_4NO_3, NH_4SO_4, NH_4Cl

Basic medium

1. KH_2PO_4	1.0g
2. $MgSO_4.7H_2O$	0.5g
3. Glucose	10.0g
4. Agar	15.0g
5. Distilled water	1 litre

All nitrogen sources were used at 0.1g/l. Autoclaved media were adjusted to pH 3.8. 6 replicates for each treatment were used. Cultures were incubated at 20°C, and colony diameters were measured after 3, 5, 7, 9, 11, 13, 15, 17 and 19 days.

3.5.4.9. Organic nitrogen nutrition

The following amino acids: tryptophane, glycine, histidine, asparagine, valine, threonine, leucine, serine, arginine, tyrosine, proline, γ-amino butyric acid, alanine, phenylalanine, cystine, aspartic acid, *iso*-leucine, and glutamic acid were added to the basic medium at 0.1g/l.(see inorganic nitrogen). Amino acids were prepared in solution then filter-sterilized separately, and then added to autoclaved basic medium. It was important to autoclave the nutrients separately since the autoclaving of amino acids and sugars together has been shown to result in the inactivation of some amino acids (Evans and Butts, 1949). Media were adjusted to pH 3.8 before dispensing them into Petri-dishes. Cultures were incubated at 20°C. and, colony diameters were measured after

10 and 15 days. Amino acid solutions were filter-sterilized and then were added to autoclaved media at 0.1g/l. Media were adjusted to pH 3.8. Cultures were incubated at 20 C and, colony diameters were measured after 5, 10 and 15 days. Aspartic acid, concentrations 0.00, 0.05, 0.07, 0.08, 0.09, 0.10 and 1.0% were tried. Preparation of media, adjustment of pH, inoculation, and incubation were carried out as described above. Colony diameters were measured after 20 days.

3.6. Data handling and analysis

Differences between: (1) body length, headcapsule widths of pupae and adult *Hylecoetus* ; (2) elytron length of females and males; and (3) numbers of eggs laid by females from natural populations and those reared in the laboratory were compared using a *t - test* (Presto package, Department of Forestry and Natural resources, University of Edinburgh). Data on emerging and flying *Hylecoetus* were analysed by establishing the relationships between: (1) numbers of emerging and flying adults; and (2) emergence, flight and weather parameters, using correlation and regression analysis (Presto and Minitab packages). The output included correlation matrices, regression equations and analysis of variance (ANOVA) comparing the regressions. The regression equation was used as a *function* in the Easygraph package (Ediburgh Regional Computing Centre) to draw the regression line in graphical presentations. All graphs were drawn using Easygraph. Data in the form of percentages were subjected to the arcsine transformation before analysis (Coster, Payne, Edson and Hart, 1978). Results in the form of small, whole-numbered counts and zeros were subjected to $\sqrt{x+0.5}$ transformations before analysis (Blight, King, Wadhams and Wenham, 1979; Parker, 1983).

RESULTS

CHAPTER 4

THE LIFE HISTORY OF *H. DERMESTOIDES*

In common with other Coleoptera, *Hylecoetus* displays complete metamorphosis i.e. the life cycle consists of egg, larva, pupa and adult (imagine).

4.1. Egg

The egg in *H. dermestoides* is whitish-yellow with a smooth shiny and creamy surface and has a soft chorion, the general shape being illustrated in Fig:14. The egg is slightly ovoid making one side (convex) longer than the concave side. A side view shows a shallow groove running along the length of the convex surface.

Length and diameter of the egg were measured as indicated in Fig:14. A sample of 83 eggs were measured using a travelling microscope. Eggs are typically 1.09±0.14 mm long by 0.35±0.04 mm diameter (Table 11).

4.2. Larva

4.2.1. Number of instars in field-collected *H. dermestoides* larvae

A *H. dermestoides* larva possesses a well-developed headcapsule. Measurements of headcapsule widths and other sclerotized parts of a random population of larvae were carried out with a view to ascertain whether Dyar's rule could be applied to *Hylecoetus* ; using plots of frequency distributions of measurements of different dimensions to estimate the number of larval instars.

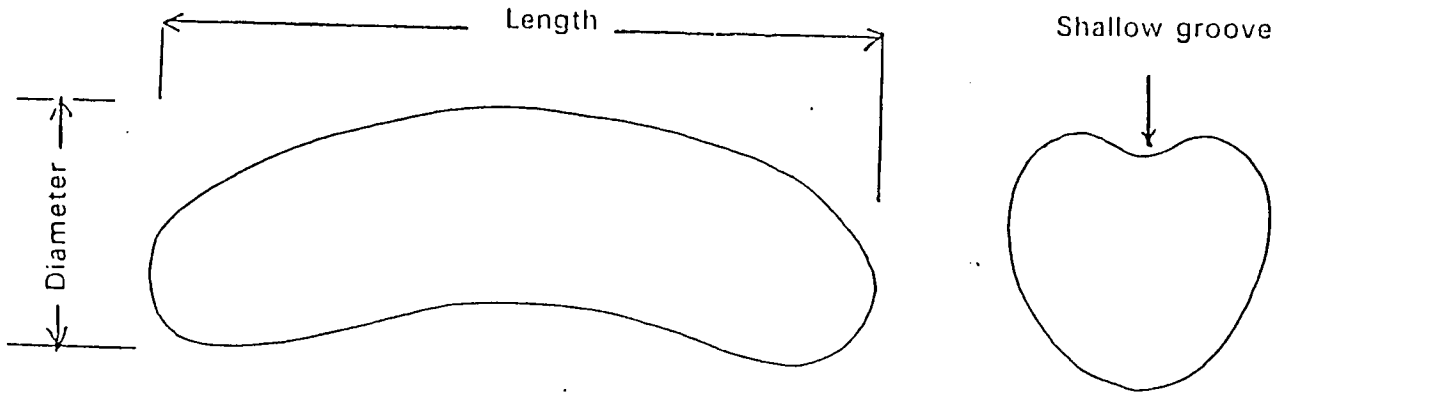


Fig: 14 Egg of *Hylecoetuss dermestoides* (a) showing where measurements of length and diameter were taken; (b) cross-section view showing shallow groove on convex side.

Table 11: COMPARISON OF LENGTH AND DIAMETER OF *Hylecoetus* EGGS COLLECTED ON THE ATHOLL ESTATE DURING THE 1984 SEASON

Dimension	Mean±SD (mm)	CV%	Min-Max
Length	1.09 ±0.14	13.64	0.88-1.60
Diameter	0.35 ±0.04	12.09	0.24-0.44

In April, 1984, sixty *Hylecoetus* larvae were collected from infested larch stumps on the Atholl Estate. The larvae were preserved in 70% ethanol before measuring various head morphological characters (Thomsen, 1950). This worker produced a general grouping of *H. dermestoides* larvae and classified them as follows: (1) first stage larvae; (2) middle instars; and (3) last instars. He did not quantify the number of instars representing the middle category. The results of the grouping of the 60 *Hylecoetus* larvae collected in 1984 into instars are presented in Table 12. Four broad groups or instars were recognized.

Table 12: INSTARS OF *Hylecoetus* LARVAE BASED ON HEAD MORPHOLOGICAL CHARACTERS OF 60 LARVAE COLLECTED ON THE ATHOLL ESTATE IN 1984.

INSTAR	NUMBER OF LARVAE	DESCRIPTION OF LARVAL INSTAR ACCORDING TO THOMSEN *et al.*(1949) AND THOMSEN (1950)
I	0	First stage larva with a horny shield on the ninth abdominal segment and has 5 ocelli.
Middle instars		
II	22	Larva has a 'tail' but ocelli or imaginal eyes are wanting.
III	19	Larva showing the beginning of the development of the imaginal eyes. The eyes are indicated by light-brown spots on each side of the headcapsule. On the front of the head, the cuticle has few small light brown spots. These brown spots are wanting in the second instar.
Last IV	19	On the front of the headcapsule the cuticle is dark brown and appears much darker than in the III instar. There is a brown crescent-shaped cuticular ridge on the side of the head, and behind this the imaginal eye visible through the semitransparent larval cuticle as a dark spot. The cuticular ridge is lacking in the III instar. The IV instar resembles Thomsen's (1950) last instar.

In October/November, 1984, 97 *Hylecoetus* larvae were collected from stumps on the Atholl Estate, and attempts made to group them into instars according to the descriptions given in Table 12. In addition to 'qualitative' characters of the headcapsule, other possible separators were considered as follows: (1) headcapsule width; (2) body length (without the 'tail'); (3) 'tail' length; (4) body plus 'tail' length; and (5) % 'tail' length/body length plus 'tail' length. A summary of the measuments is given in Table 13; and frequency distributions of the headcapsule, body and 'tail' measurements are illustrated in Figs 15, 16, 17, 18 and 19. None of the measurements fell into distinct groups. The range of measurements of each dimension (Table 13) and the plots of histograms (Figs 15–19) clearly show that the range of variation of the dimensions among individuals of the instars overlap those of the next instar, and hence it is not possible to determine the number of larval instars from such measurements.

Dyar's ratio of increase is derived by the division of an actual measurement by the measurement of the stage immediately preceding it. In Table 13, the growth ratio is obtained by a division of the mean headcapsule width of the second instar by that of the first, the third by the second and the fourth by the third. The mean of the observed growth ratios is then used as a common ratio for calculating a progression beginning with the first or last term of the observed progression. The mean growth ratio multiplied by the mean headcapsule width of the first instar gives the mean headcapsule width of the second instar; or the mean headcapsule width of the last instar divided by the mean growth ratio gives the mean width of the preceding instar etc. The growth ratio of the headcapsule width between the II/I instars was 3.46, III/II, 1.38 and IV/IIII was 1.14 respectively. The three growth ratios, 3.46, 1.38 and 1.14 were used to calculate an average ratio, 1.99.

Due to the small number of first stage larvae collected, attempts were

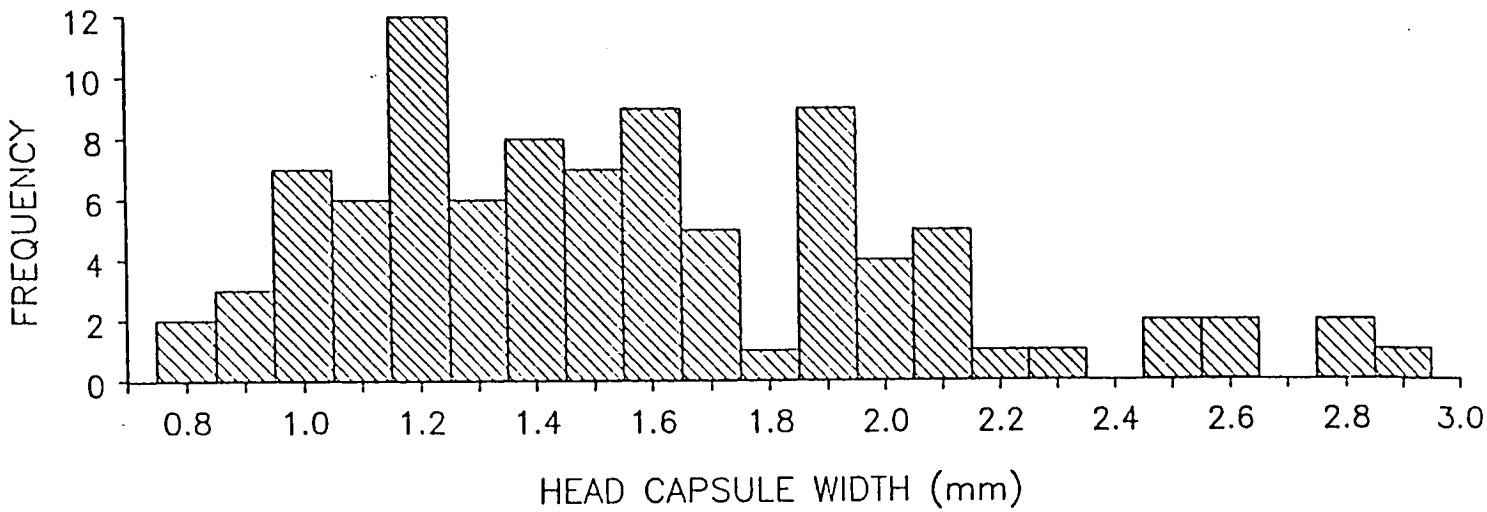


Fig:15 Frequency distribution of headcapsule widths of *H. dermestoides* larvae from naturally infested *Larix decidua* stumps on the Atholl Estate in 1984 (n=93).

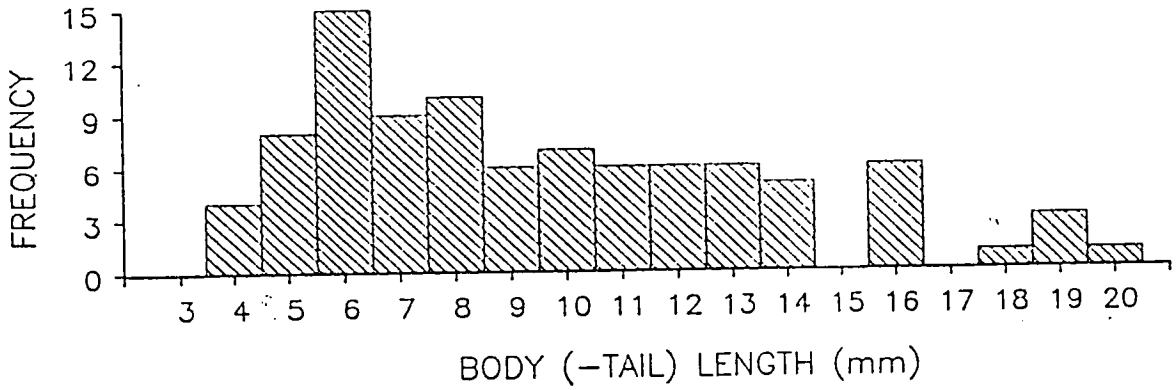


Fig:16 Frequency distribution of total body (+ tail) lengths of *H. dermestoides* larvae from naturally infested *Larix decidua* stumps on the Atholl Estate in 1984 (n=93).

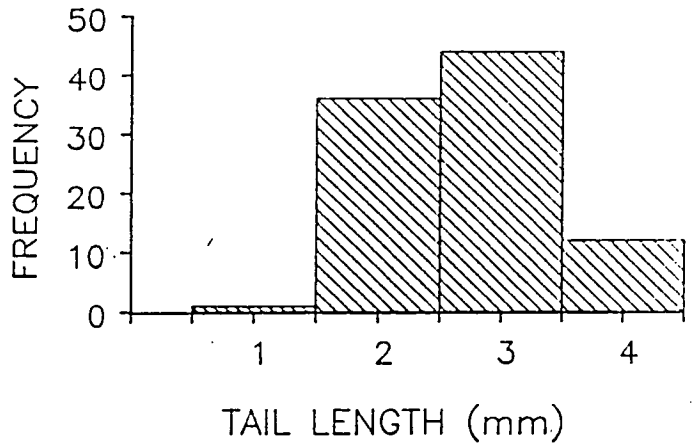


Fig:17 Frequency distribution of tail lengths of *H. dermestoides* larvae from naturally infested *Larix decidua* stumps on the Atholl Estate in 1984 (n=93).

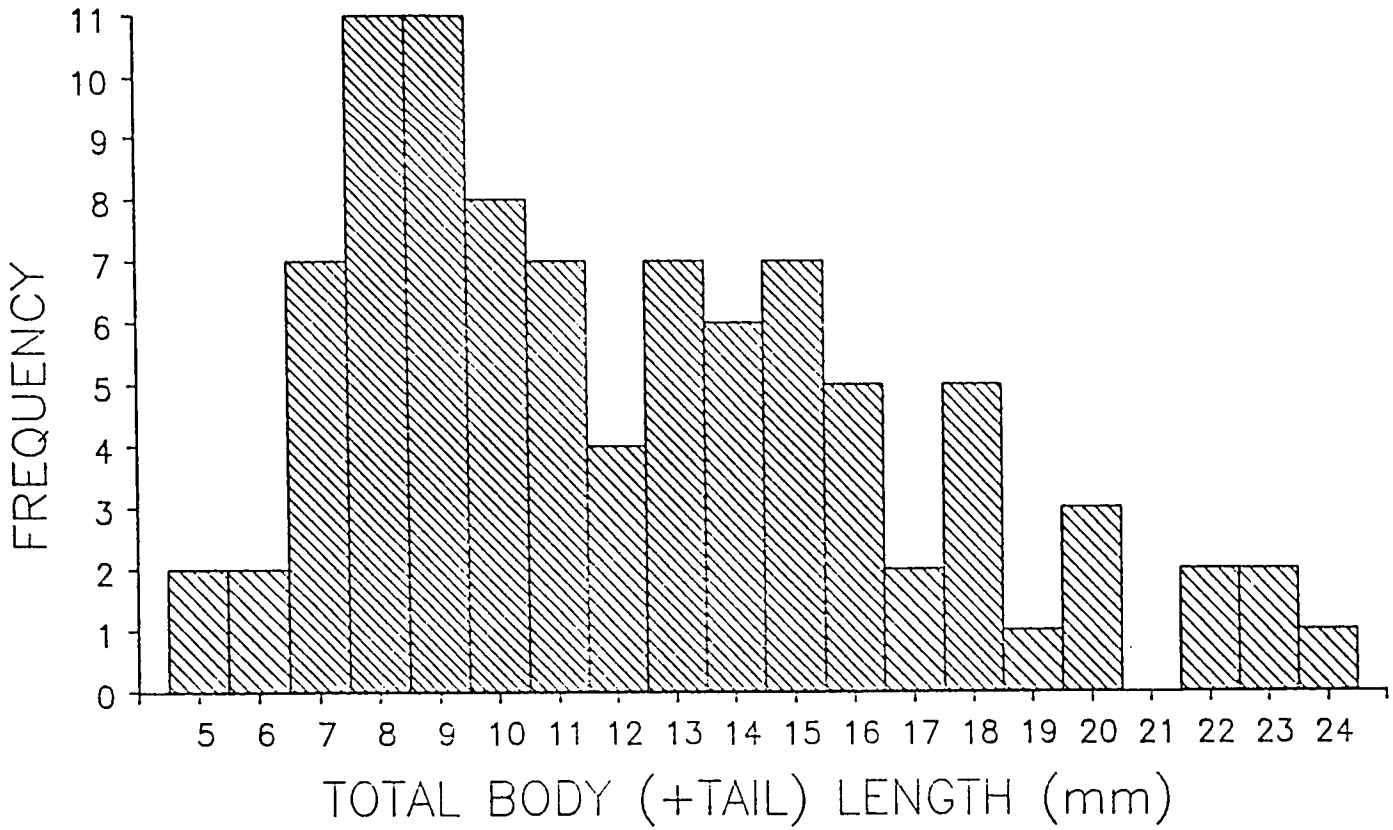


Fig:18 Frequency distribution of total body(+tail) lengths of *Hylecoetus dermestoides* larvae from naturally infested *Larix decidua* stumps on the Atholl Estate in 1984 (n=93).

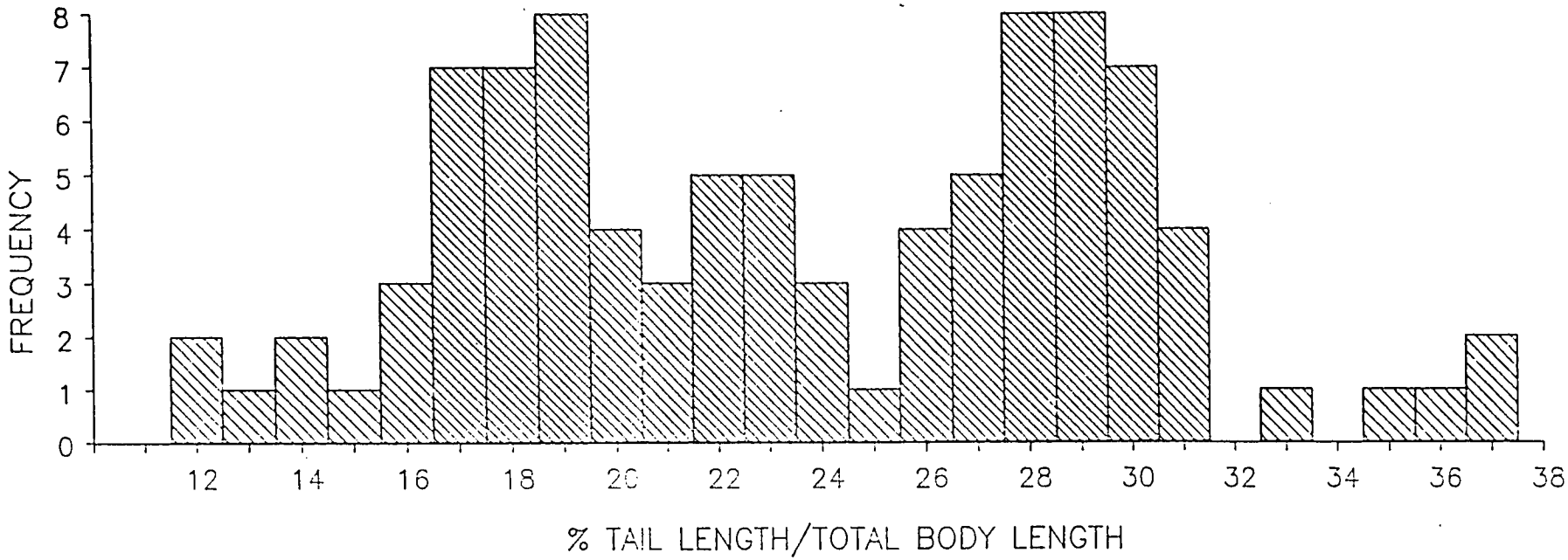


Fig:19 Frequency distribution of tail length as percentage of total body length of *Hylecoetus dermestoides* larvae from naturally infested *Larix decidua* stumps on the Atholl Estate in 1984 (n=93).

Table 13 : SUMMARY OF MEASUREMENTS OF *Hylecoetus* LARVAE COLLECTED IN OCTOBER/NOVEMBER, 1984 ON THE ATHOLL ESTATE

Dimension	Instar	Mean±SD (mm)	CV%	Range	Number of larvae	Growth ratio
Head capsule width	I	0.37±0.02	15.67	0.34-0.40	4	3.45
	II	1.28±0.28	22.26	0.76-2.04	52	1.38
	III	1.77±0.45	25.82	1.24-2.88	15	1.14
	IV	2.02±0.40	20.09	1.12-2.84	26	-
					Mean	1.99
Body minus 'tail' length	I	-	-	-	4	-
	II	7.10±2.37	33.40	3.56-13.58	52	1.49
	III	10.64±3.71	34.91	5.88-19.20	15	1.29
	IV	13.74 ±3.19	23.22	8.00-19.78	26	-
'Tail' length	I	-	-	-	4	-
	II	2.63 ±0.66	25.29	1.52-4.3	52	0.17
	III	2.53 ±0.59	23.43	1.92-4.3	15	1.13
	IV	2.88±0.65	22.72	1.08-4.12	26	-
Body plus 'tail' length	I	2.16±0.20	9.711	1.98-2.42	4	4.51
	II	9.74 ±2.92	30.0	5.2-17.38	52	1.35
	III	13.17 ±4.05	30.78	8.34-21.7	15	1.26
	IV	16.63 ±3.74	22.54	9.08-23.60	26	
%'tail' length/ total body	I	-	-	-	4	-
	II	27.62±4.07	14.76	19.19-37.36	52	0.72
	III	20.15 ±4.67	23.18	11.52-29.49	15	0.86
	IV	17.43 ±2.27	13.05	11.89-21.54	26	-

made to use the technique of MacLean and Giese (1967). These workers determined the headcapsule dimensions of the first instar larvae by dissecting well-developed eggs and newly hatched larvae of *Xyloterinus politus* . They found that the headcapsule width from egg dissections never exceeded 0.46mm.; and mean width of the headcapsule of newly hatched larvae was 0.44mm. Eighty three *Hylecoetus* eggs were examined using this technique and the results presented in Table 11, the mean diameter of the egg being

0.35±0.04mm.

The average growth ratio 1.99 and the average headcapsule width of the last instars were used to determine predicted Dyar's (theoertical) headcapsule widths of the different larval instars. Using a growth ratio of 1.99 reveals 4 instars between 0.4 and 2.02 mm. (Table 14)

Table 14: ACTUAL AND THEORETICAL HEADCAPSULE MEAN WIDTHS FOR DIFFERENT INSTARS OF *Hylecoetus* LARVAE

Instar	Actual (mm)	Theoretical measurements	Difference %
I	0.37	0.25	32.43
II	1.28	0.50	60.93
III	1.77	1.01	42.93
IV	2.02	2.02	-

To confirm the suggestion by MacLean and Giese (1967), more headcapsules of *Hylecoetus* were measured with a view to represent the entire larval stage. A total of 376 headcapsule measurements were obtained, and a plot of the histogram of the measurements and the 83 egg diameters is illustrated in Fig:20. From Fig:20 two interesting points arise: (1) the failure of the measurements to fall into distinct groups; and (2) the small number of larvae with headcapsules between 0.4 and 0.7 mm. The measurements of the headcapsules do not fall into well defined groups which could be taken to indicate the modes of given instars. Every size from 0.3 to 2.88 mm. with intervals of 0.1 mm. occurs except 2.7 mm. This can be taken as evidence that the headcapsule is not a constant size in any one instar, but that it varies within limits, confirming the conclusion from Figs 15-19, i.e. that the limits of

instars overlap.

It is possible that males and females in *Hylecoetus* may be differentiated in the larval stage by headcapsule size. The measurements of 40 headcapsules of male and female pupae (sex ratio 1♀:♂1) shown in Fig:21 and Table 17 clearly demonstrate that the headcapsules are of different sizes in the two sexes. The two peaks at 2.0 and 2.4 mm. correspond to the mean headcapsule widths for male and female pupae. Fig:22 shows the results of headcapsule measurements of a random sample of older larvae (only larvae with eyes and those with last instar characters) which show peaks at 1.2, 1.5, 1.9 and 2.1 mm. These modes probably identify the last two larval instars with 1.2 and 1.9 mm.; and 1.5 and 2.1 mm. being modes for males and females respectively. In this case, in males the last two instars would have a growth ratio of 1.58; and female larvae would have a growth ratio of 1.4. Therefore, in applying Dyar's rule, growth ratios 1.58 and 1.4, and 1.90 and 2.10 mm. as mean headcapsule widths for last instars of male and female larvae, and mean width of the headcapsules of the 4 first stage larvae, reveals a minimum of 5 instars for male and female larvae (Table 15). Fig:20 appears to indicate that differentiation of male and female larvae on the basis of headcapsule size is evident in older instars. However, there is so much overlap in the ranges of headcapsule widths of female and male larvae that it is impossible to separate the modes for female and male larval instars. Distribution of egg size indicates that there are two distinct groups at 0.3 and 0.4 mm., and it seems that the smaller eggs (0.3 mm. diameter) would give rise to males whereas the larger eggs would produce females.

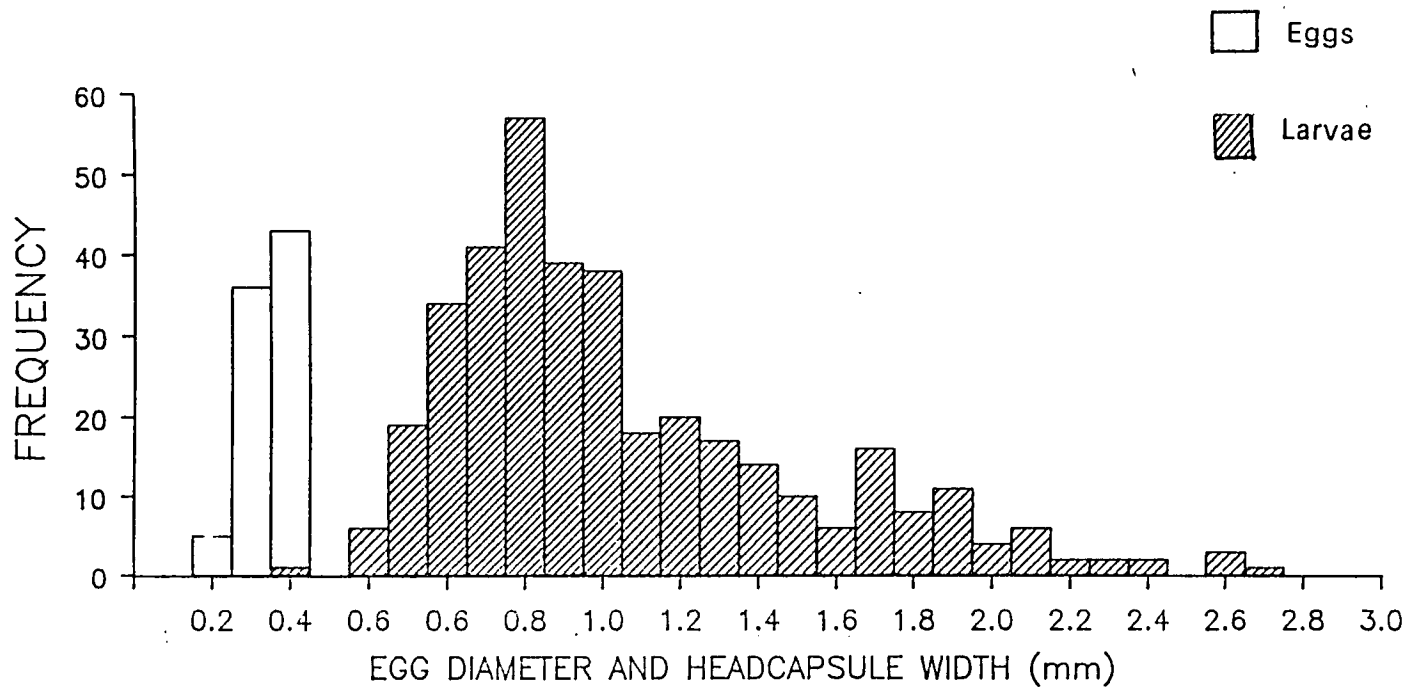


Fig:20 Frequency distribution of headcapsule widths of *Hylecoetus dermestoides* larvae (n=376) and 83 eggs collected from the Atholl Estate in 1984-1986.

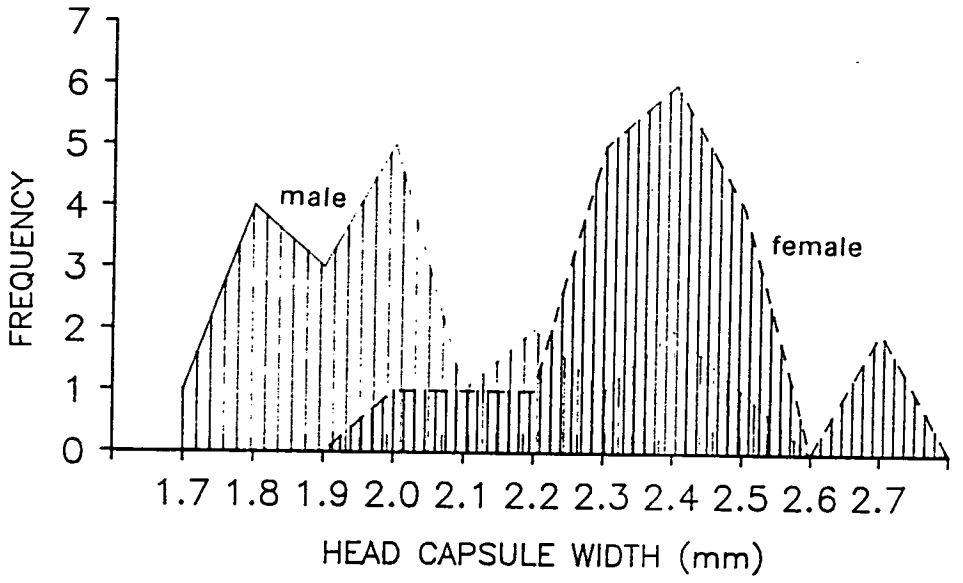


Fig: 21 Frequency distribution of headcapsule widths of female and male *Hylecoetus dermestoides* pupae collected from naturally infested *Larix decidua* stumps on the Atholl Estate in 1984-1986 (n=40).

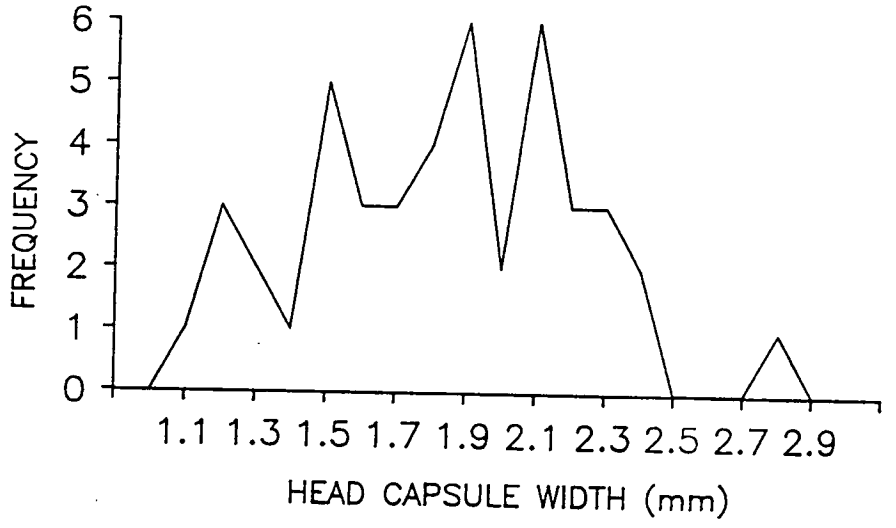


Fig:22 Frequency distribution of headcapsule widths of *Hylecoetus dermestoides* older larvae collected from naturally infested *Larix decidua* stumps on the Atholl Estate in 1984-1986 (n=45).

Table 15: CALCULATION OF MEANS FOR INSTARS OF *Hylecoetus* LARVAE BY APPLYING DYAR'S RULE AND USING DIFFERENT GROWTH RATIOS FOR FEMALE AND MALE LARVAE COLLECTED ON ATHOLL ESTATE IN 1984/85.

Instar	Mean head capsule width		Remarks
	Male (mm)	Female (mm)	
I	0.303	0.54	Theoretical measurements,
II	0.480	0.76	with lower limit fixed by
III	0.759	1.07	size of first stage larvae and eggs.
IV	1.20	1.50	Actual measurements
V	1.90	2.10	

(Males: growth ratio = 1.58; Females: growth ratio = 1.4)

The means of the 5 larval instars for male and female larvae are shown in Table 15.

From a comparison of the results in Figs 21 and 22 and Tables 14 and 15, it appears to be a reasonable suggestion that males and females are already of differential sizes in the larval stage. It also follows that larvae with 'tails' but without eyes do not all belong to the same instar in contrast to the data in Table 12. Table 15 confirms that such larvae belong to at least two instars. Direct observation of larvae on agar showed that some larvae without eyes would still not have eyes after moulting. Therefore, the 'qualitative' headcapsule characters combined with the application of Dyar's rule and the plotting of frequency distributions of headcapsule measurements of random populations representing female and male *Hylecoetus* larvae are not reliable methods to use when determining the number of *Hylecoetus* larval instars. However, the latter methods would be useful if female and male larvae could be separated at the pupal stage.

4.2.2. Moulting by *Hylecoetus* larvae reared on agar at 20°C.

Second instar larvae of *H. dermestoides* (without eyes) (Table 12) or second/third instars (Table 14) were collected on the Atholl Estate and then reared individually on agar media in the laboratory under controlled conditions (20°C.). Observations were made on the larvae from the end of September, 1985 to the end of May, 1986. A total of 81 moults were recorded and the distribution of the moults in relation to the time of the year is illustrated in Fig:23. Information on moulting by 8 specific larvae which reached the pupal and adult stages respectively was obtained. The 8 (A-H) larvae moulted once during the following periods: (A), 1-9 October and 26 October - 4 November; (B) 17-25 October and 26 October - 4 November; (C) 26 October - 4 November and 5-11 November; (D) 5-11 November and 16 November - 3 December; (E) 5-11 November and 16 November - 3 December; (F) 16 November - 3 December and 4-16 December; (G) 17 December - 10 January, 1986 and 28, January - 14 February; a and (H) 21-31 March and 1-23 April. The other 65 moults were by larvae which died before they reached the pupal stage. The 8 (A-H) larvae had two moults each before reaching the pupal stage. According to Table 15, this would give evidence that larvae were third instars at the time of collection, and therefore they had a total of 5 instars. In addition, after the first moult by each of the 8 larvae, the beginning of the development of the eyes was observed, and all the 8 larvae became last instars after the second moult. After May, 1986 larvae without eyes and larvae with eyes but not last instars remained alive on agar.

There was virtually no moulting by larvae between December, 1985 and January, 1986. This period seems to indicate the stage of overwintering by *Hylecoetus* larvae. Seven of the 8 larvae had become last instars before January, 1986, whereas the other single larva became a last instar in

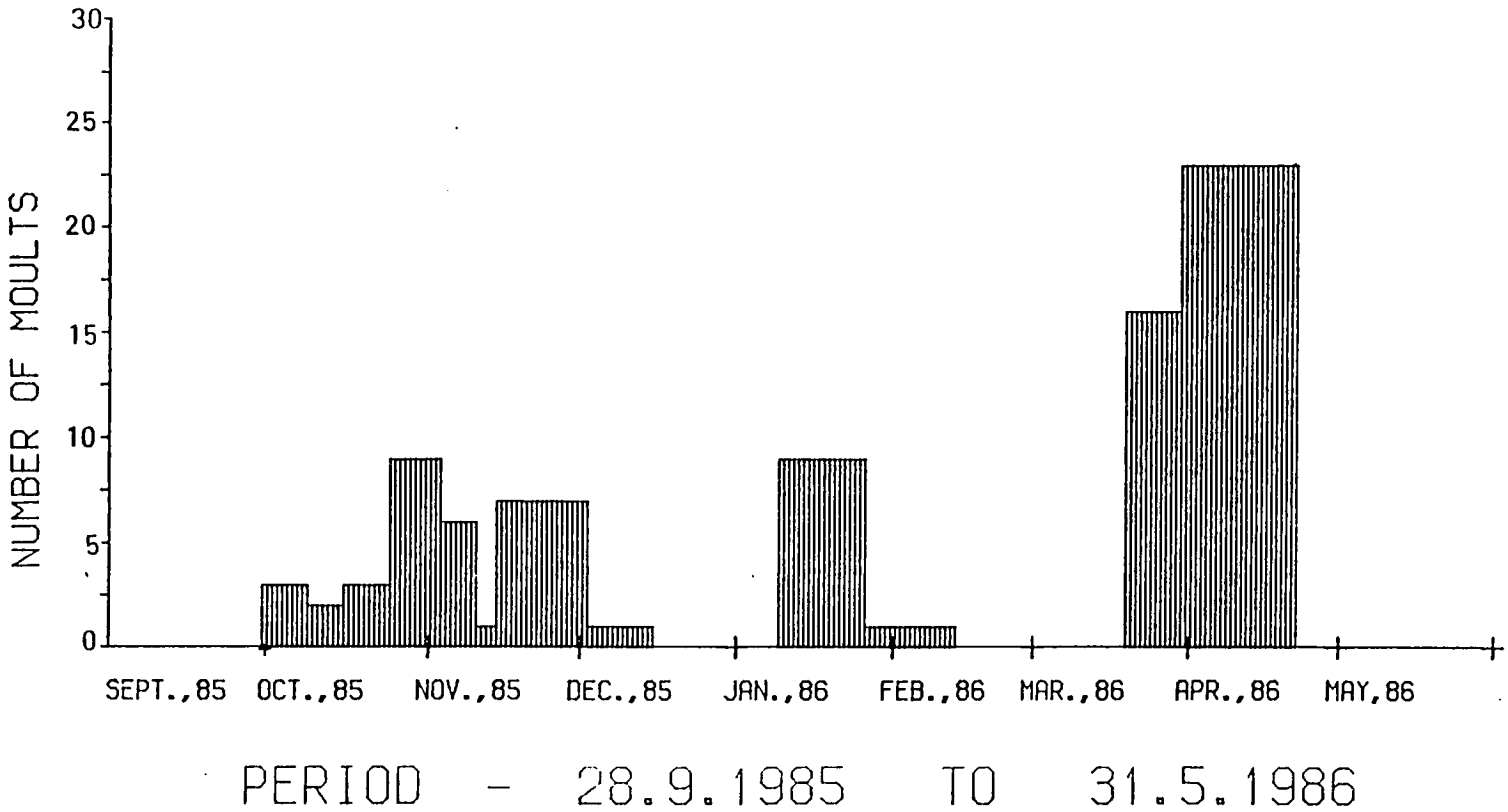


Fig: 23 Moulting by *Hylecoetus dermestoides* larvae kept on agar at 20°C.

February, 1986. The moulting taking place between January and February seems to indicate the spring feeding activity by the larvae. Fig:23 shows that the overwintering period may be passed through by II, III and IV larval instars. However, most of the larvae which are due to pupate would become last instars before the onset of winter. Pupation was first recorded on 4 March, 1986. Larvae had approximately an average of 25.6±6.6 days with a range of 14 to 32 days between 2 moults when reared on agar at 20°C.

4.3. The pupa

Pupation was observed by examining last instar larvae kept on agar at 20°C. The pupation period ranged from 6 to 8 days with an average of 7.2 days (Table 16). Prior to emergence of adult *Hylecoetus*, it was possible to separate the pupae into two groups according to the sexes of the emerging adults. Newly emerged pupae are pale white. However, the pupae later turn black or brown, black pupae being male, while brown pupae are female. Size variation of black (male) and brown (female) pupae from naturally infested stumps of felled larches on the Atholl Estate was investigated by measuring the size of the body and the headcapsule width. Within and between sex variation was analysed and the results are summarised in Table 17. Female pupae are significantly larger than males (p = 0.01). Female pupae, in addition, have significanlty larger headcapsules than males (p = 0.01).

The relationship between body length and headcapsule width for females and males is illustrated in Fig:24.

Table 16: RESULTS OF OBSERVATIONS ON PUPATION OF *Hylecoetus* LARVAE KEPT ON AGAR AT 20°C.

No	Date of emergence of pupae (days)	Duration of pupation (days)	Adult emerged
1	4.3.1986	7	Male
2	6.3.1986	7	Female
3	7.3.1986	6	Female
4	11.3.1986	7	Female
5	22.3.1986	7	Female
6	24.3.1986	8	Female
7	24.3.1986	8	Female
8	30.3.1986	8	Male
9	8.4.1986	7	Female
10	14.4.1986	7	Female
	Mean	7.2	

Table 17: COMPARISON OF BODY LENGTH AND HEADCAPSULE WIDTH OF MALE AND FEMALE PUPAE OF *Hylecoetus* COLLECTED FROM NATURALLY INFESTED STUMPS OF FELLED LARCHES ON ATHOLL ESTATE IN 1984

Dimension	Sex	Mean±SD (mm)	CV%	Min-Max	T-Value	Df	Probability ($p=$)
Body length	Males	12.04±1.68	14.02	9.1 — 15.7	8.10	19	0.001
	Females	16.45 ±1.75	10.66	13.6 — 19.4			
Headcapsule width	Males	2.03 ±0.23	11.32	1.7 — 2.5	5.38	19	0.001
	Females	2.38±0.17	7.15	2.0 — 2.7			

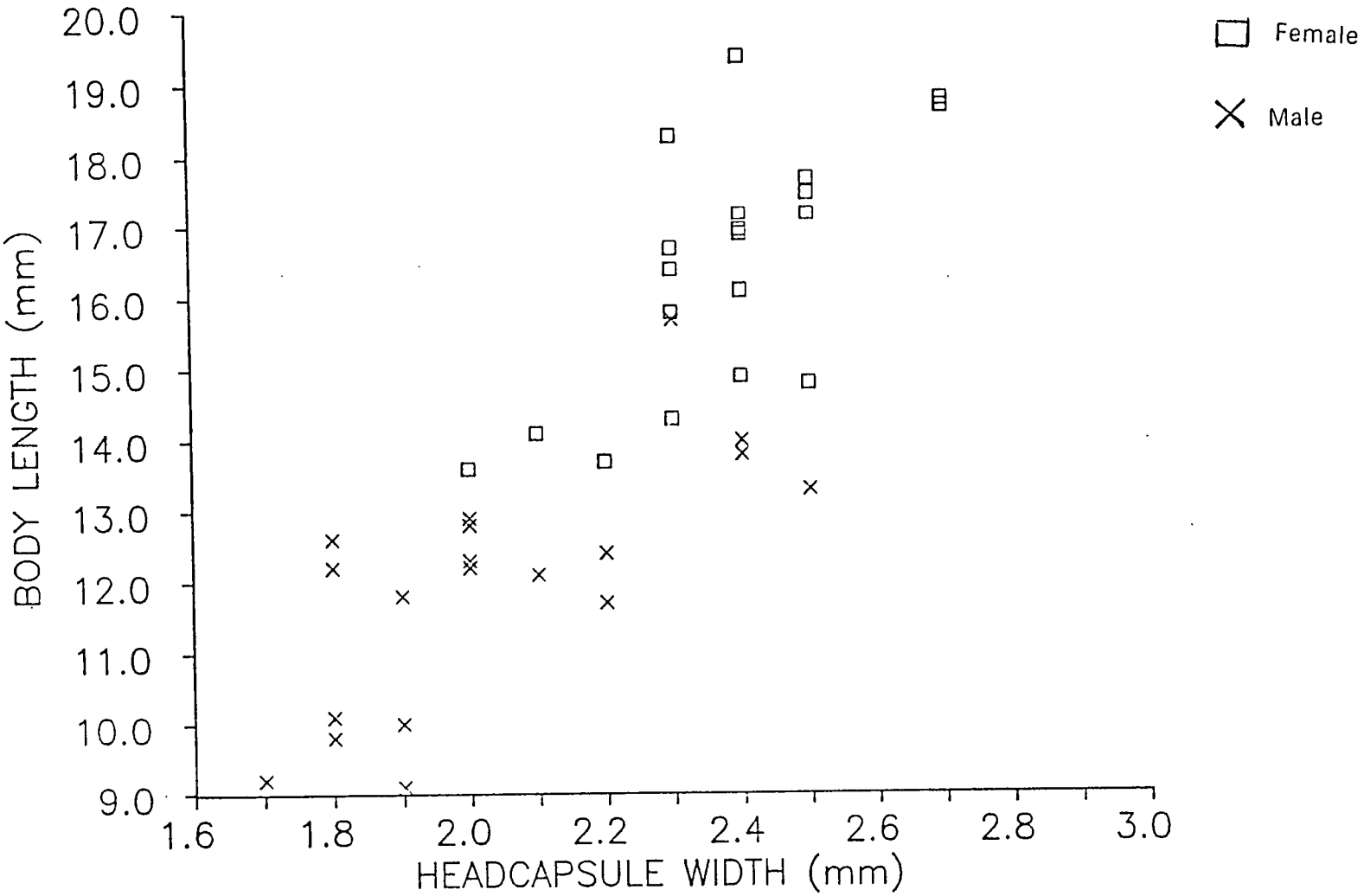


Fig: 24 Relationships between the headcapsule width and body length of *Hylecoetus dermestoides* pupae from naturally infested *Larix decidua* stumps on the Atholl Estate in 1984.

4.4. Adult

4.4.1. Sexual dimorphism

After pupal ecdysis, female and male *H. dermestodes* were distinguished using the characters described by Fergusson (1920). Both all-black males and yellowish, brown males were collected (Fig:25a). A summary of collections of adult *Hylecoetus* from the Atholl Estate made in 1894-85 is given in Table 18.

Table 18: DISTRIBUTION OF MORPHOLOGICAL TYPES AND SEX RATIOS OF ADULT *Hylecoetus* COLLECTED ON THE ATHOLL ESTATE IN 1984-1985

SEX	1984		1985	
	Emergence box	Flight	Pre-emergence	Flight
Female	25	225	62	19
Male	25	93	76	2
Total	50	318	138	21
Sex ratio (♀:♂)	1:1	2.4:1	1:1.2	9.5:1
All-black Males	22	82	69	2
Brown Males	3	11	7	0
Total	25	93	76	2
Male ratio (Black:Brown)	7.3:1	7.4:1	10:1	-

4.4.2. Comparison of adult *Hylecoetus* collected on the Atholl Estate during the 1984-1986 seasons

The distribution of adult *Hylecoetus* from natural populations on the Atholl Estate was investigated by examining females and males collected from: (1) caged naturally infested pieces of larch wood; (2) cut stumps in the field prior to flight; and (3) *Hylecoetus* responding to different baits in flight traps.

4.4.2.1. Pre-emergence and emergence boxes

Fifty adult *Hylecoetus* were collected from the emergence boxes in 1984 (sex ratio ♀1:♂1). More all-black than yellowish brown males were collected, with a ratio of black to brown of 7.3:1. 138 adult *Hylecoetus* were collected from naturally infested *Larix decidua* stumps prior to emergence in 1985. The teneral adults had a sex ratio of ♀1:1.2♂. The 76 males had approximately a black to brown ratio of 10:1. There do not seem to be differences between the 1984 and 1985 seasons in the sex ratio. This indicates that more or less equal numbers of female and male *Hylecoetus* may emerge from the pupae. However, within the male sex, there will be more all-black than yellowish brown forms.

4.4.2.2. *Hylecoetus* in flight

More female than male *Hylecoetus* were collected from flight traps in 1984 (sex ratio ♀2.4:1♂) and more all-black than yellowish brown males were collected with a black to yellowish brown ratio of 7.4:1. There was no difference between emerging and *Hylecoetus* in flight in the male ratio in 1984. 21 *Hylecoetus* were collected from flight traps in 1985 (sex ratio ♀9.5:1♂) (Table 18).

4.4.2.3. Size variation in adult *Hylecoetus*

In adult *Hylecoetus* the body length and elytron length differed between males and female. On average females are significantly larger than males (Fig:25b). Females have significantly longer elytra than males (Table 19). The ratio of elytron length to total body length is the same for both females and males (Table 20). The size variation between emerging *Hylecoetus* and *Hylecoetus* caught in flight; and the variation between all-black and yellowish brown males was not investigated. Table 19 also shows comparisons between pupae and adult *Hylecoetus*. Both female and male pupae are larger than the emerging adults.

Fig:25. (a) All-black and yellowish brown male *Hylecoetus dermestoides*; and (b) male (black) and female (brown) *H. dermestoides* collected on the Atholl Estate in 1984.

Table 19: SIZE VARIATION IN PUPAL AND ADULT *Hylecoetus* FROM NATURALLY INFESTED STUMPS OF FELLED LARCHES ON ATHOLL ESTATE

Dimension	Growth stage	Mean±SD (mm)	CV%	Min-Max (mm)	T-Value	Df	Probability (p =)
Elytron length	Adult Females	10.60±1.33	12.57	8.00-12.5	4.625	19	0.001
	Adult Males	8.86±1.027	11.59	6.70-10.5			
	Adult females	13.27±1.68	12.68	10.30-16.4	4.881	19	0.001
	Adult males	11.00±1.22	11.04	8.20-12.7			
	Adult females	13.27±1.68	12.68	10.30-16.4	5.8646	19	0.001
	Female pupae	16.45±1.76	10.67	13.60-19.4			
Body Length	Adult males	11.00±1.22	11.04	8.20-12.7	2.235	19	0.05
	Male pupae	12.04±1.69	14.02	9.10-15.7			

Table 20 RELATIONSHIPS BETWEEN ELYTRON LENGTH AND BODY LENGTH OF FEMALE AND MALE *Hylecoetus dermestoides* COLLECTED ON THE ATHOLL ESTATE IN 1984

No	Body length (mm)	Elytron length (mm)	Elytron length/ Body length (%)	Body length (mm)	Elytron length (mm)	Elytron length/ Body length (%)
1	13.0	10.4	80.00	11.2	9.2	82.14
2	16.4	11.9	72.56	11.5	8.9	77.39
3	15.1	12 0	79.47	11.9	9.7	81.51
4	14.6	11.5	78.77	11.9	10 5	88.23
5	11.2	8.9	79.46	10.4	8.6	82.69
6	14.0	11.1	79.29	9.7	7.7	79.38
7	12.5	9.7	77.60	9.2	7.8	84.78
8	12.4	10.0	80.64	10.6	8.9	83.96
9	10.9	8.8	80.73	10.6	9.4	88.68
10	14.0	10.9	77.86	12.4	10.1	81.14
11	14.9	11.7	78.52	11.0	9.6	87.27
12	12.9	10.2	79.07	11.0	8.7	79.09
13	10.3	8.0	77.67	12.7	10.1	79.52
14	12.9	10.8	83.72	12.1	9.8	80.99
15	14.8	12.2	82.24	11.7	8.3	70.94
16	14.7	12.5	85.03	12.1	9.0	74.38
17	12.9	10.7	82.94	11.2	8.3	74.10
18	10.5	8.4	80.00	8.9	6.9	77.52
19	14.7	12.1	82.31	8.2	6.7	81.70
20	12.6	10.2	80.95	11.7	9.0	76.92
		Mean SD(%)	79.94 2.68		Mean SD(%)	80.62 4.6

4.4.3. Pairing

A summary of observations on pairing (mating) by adult *Hylecoetus* under natural conditions on the Atholl Estate is shown in Table 21. All mating pairs were collected between 13.00 and 17.00 HRS.

A total of 13 pairs of mating *Hylecoetus* were recorded in 1984 (Table 21); 8 of the pairs were collected from the surfaces of standing dead larches and 5 from flight traps.

The diurnal mating pattern for *Hylecoetus* closely resembles its diurnal flight pattern for both males and females. It can be seen that mating occurred during the peaks for diurnal flight. The largest number of pairs (5 of 13 pairs captured) was collected at 15.00 HRS.

4.4.4. Oviposition

An inventory of oviposition by *Hylecoetus* on the Atholl Estate during the 1984 season is shown in Table 22. Oviposition occurred throughout the flight season. Of female *Hylecoetus* from natural populations, 31 produced eggs and most oviposition occurred during the peaks of seasonal flight (Fig:26); 7 of 31 ovipositing females laid eggs on the day they were collected from the trapping sites, and 21 of 31 females laid eggs one day after they were collected. The eggs were laid in batches and all the 31 females produced at least one batch of eggs. Six of the 31 females had 2, and 4 females had 3 batches of eggs each. The number of eggs in the first batch ranged from 4 to 45 (mean=21); the range in the second batch was 12 to 29 (mean=21) eggs; and in the third batch the range was 5 to 20 (mean=11). The mean number of batches of eggs per female was 1; mean number of eggs produced by one female was 29 and most eggs were laid in the first batch. There were no significant differences between the three batches in the mean number of eggs laid.

Table 21: NUMBERS OF PAIRS OF MATING *Hylecoetus* FOUND ON THE ATHOLL ESTATE DURING THE 1984 SEASON

Date of collection	HOUR OF DAY					Total
	13.00	14.00	15.00	16.00	17.00	
13 May	0	0	1	1	2	4
14 May	0	1	1	0	0	2
31 May	0	0	1	0	0	1
7 June	2	2	2	0	0	6
Total	2	3	5	1	2	13

Table 22: INVENTORY OF EGGS LAID BY FEMALE *Hylecoetus* CAUGHT IN FLIGHT ON THE ATHOLL ESTATE IN 1984

No.	Date of oviposition	Days between collection of adult and emergence	Eggs laid			Total Batches	Eggs per female
			Batch 1	Batch 2	Batch 3		
1	29 April	0	19	16		2	35
2	14 May	1	7			1	7
3	14	1	29			1	29
4	14	1	12			1	12
5	14	1	41			1	41
6	15	1	17	27		2	44
7	15	1	7			1	7
8	15	1	4	17		2	21
9	15	1	32			1	32
10	15	1	24	23		2	47
11	22	0	18	12	5	3	35
12	24	1	15			1	15
13	24	1	10	17	14	3	61
14	24	1	38			1	38
15	24	1	10			1	10
16	24	1	27	29	5	3	61
17	28	0	22			1	22
18	28	0	15			1	15
19	28	0	8			1	8
20	28	0	18			1	18
21	28	0	33	27		2	60
22	8 June	1	12			1	12
23	8	1	37			1	37
24	8	1	25	17		2	42
25	8	1	39			1	39
26	9	1	13			1	13
27	14	1	17	22	20	3	59
28	15	1	45			1	45
29	15	1	25			1	25
30	16	1	17			1	17
31	16	1	26			1	26
	n	31	31	10	4	31	31
	Total	24	662	207	44	45	913
	Mean	0.77	21.3	20.7	11	1.45	29.4
	SD	0.425	11.155	5.716	7.348	0.722	16.19
	CV%	55	52	27.6	66.8	49.8	55
	Min.	0	4	12	5	1	7
	Max.	1	45	29	20	3	61

Oviposition by 6 adults originating from pupae on agar at 20°C. was recorded and the results are shown in Table 23. Female *Hylecoetus* produced only one batch of eggs each with a range of 10 to 28 (mean=18) eggs per female. There were no significant differences between females from natural populations and females on agar in the number of eggs produced by each female. Females on agar on average laid eggs 1 day after emergence.

Table 23.: INVENTORY OF EGGS LAID BY FEMALE *Hylecoetus* ORIGINATING FROM PUPAE ON AGAR AT 20°C.

No.	Date adult female appeared on agar plate	Days from to oviposition	Number of eggs laid
1	29 March, 1986	2	16
2	2 April, 1986	1	14
3	2 April, 1986	1	23
4	7 April, 1986	1	10
5	15 April, 1986	1	14
6	20 April, 1986	2	28
	Mean	1.3	17.5 (18)

4.4.5. Relationship between seasonal emergence, flight, pairing and oviposition

The relationships between seasonal emergence, flight, pairing and oviposition are illustrated in Fig:26, and are summarised in Appendix 1. It is clear that these behavioural characteristics for *Hylecoetus* occur at the same time. It therefore seems likely that weather conditions influencing for instance adult emergence will indirectly affect other behaviour patterns of *Hylecoetus*.

4.4.6. Incubation and hatching

A total of 105 eggs which were produced by female *Hylecoetus* originating from pupae on agar (Table 23) were incubated at 20°C. None of the eggs hatched into larvae.

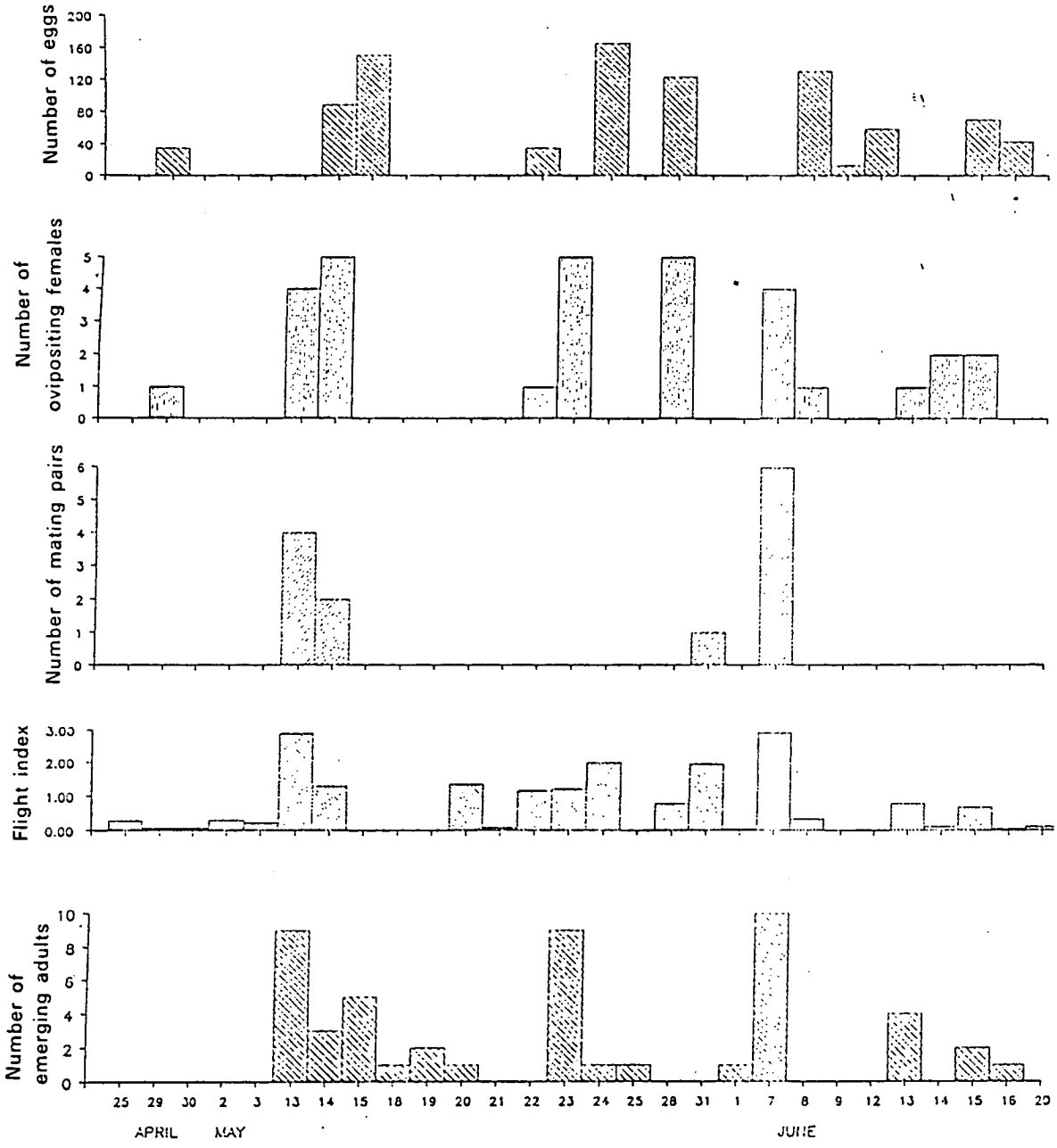


Fig:26 Relationship between emergence, flight index, pairing and oviposition in *Hylecoetus dermestoides* on the Atholl Estate during the 1984 season.

4.5. Duration of the life cycle

4.5.1. Development under natural conditions

Norway spruce stumps which were naturally infested with *H. dermestoides* in Craigvinean forest (Compartment 14, felled in February, 1983; Table 8), were examined by dissecting them with an axe, from the middle of March to the end of June during the 1984 season. Middle and last instar larvae, pupae and adult *Hylecoetus* were collected from the stumps. This shows that part of the *H. dermestoides* population requires at least one year to complete the development.

During the second week of May, 1985 standing dead larches which were used for trapping *Hylecoetus* in 1984 on the Atholl Estate, were inspected for the occurrence of *Hylecoetus*. Twenty six specimens were obtained from the dissected stumps (the trees had been felled in August/September, 1984) and they were distributed as follows: 14 larvae; 4 pupae and 8 adult males.

Part of Compartment 236 on the Atholl Estate (Map 3, Table 8) was clearfelled during August/September, 1984. Larvae, pupae and adult *Hylecoetus* were collected from dissected stumps during the 1985 and 1986 seasons and only larvae were collected from the stumps in May during the 1987 season. If all the *Hylecoetus* material collected from the stumps belonged to one generation then a 3-year life cycle would be possible. However, the larvae collected in May, 1987 were all middle instars, and this seems to indicate that *Hylecoetus* females may lay eggs in hosts which contain a brood or broods from earlier generations.

A summary of the seasonal history of *H. dermestoides* on the Atholl Estate during the 1984, 1985 and 1986 seasons is presented in Table 24 and Fig:27. During and after the 1984 flight season middle instars (II, III and IV) from a previous generation were collected from larch stumps. These larvae were

Table 24 SEASONAL HISTORY OF *Hylecoetus* ON THE ATHOLL ESTATE IN 1984-1986

YEAR	1984										1984		1985										1985		1986					
STAGE	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J
LARVAL INSTARS I																						4									
II&III											4						29	2	24			245	16		4						4
IV											21											5			18						
V			9	39						6	9	7					15	2							40		105		31		
PUPA			—	—	86	—											114	20									1		1		
ADULTS UNDER THE BARK				11													66	55										1	4		
ADULTS FROM BOXES					32	18																									
ADULTS IN FLIGHT				4	260	81											19	2													
MATING ADULTS					7	6																									
EGG LAYING					21	10																									

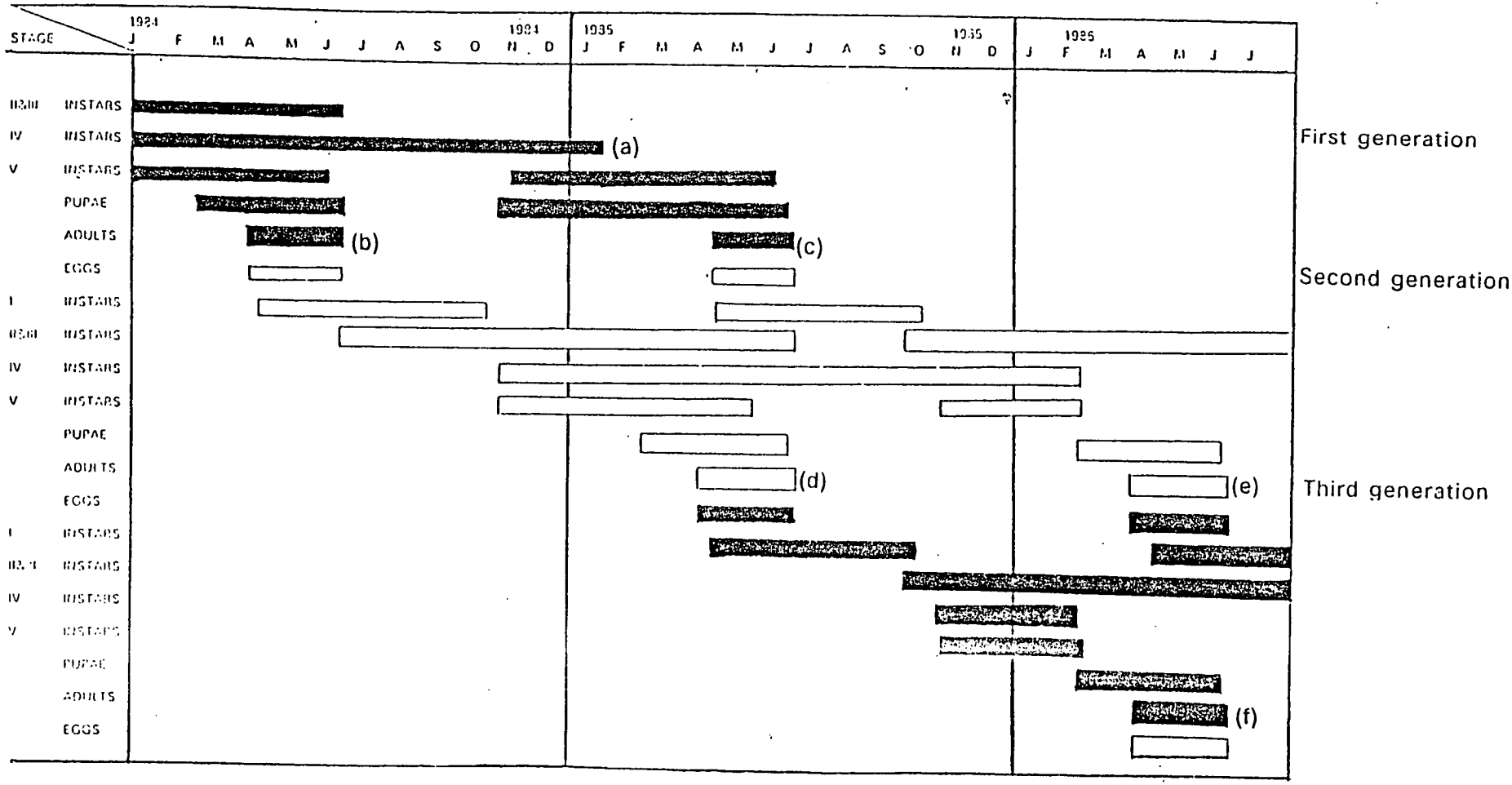


Fig:27. Periods of activity and stages of life cycle of *H. dermestoides* on the Atholl Estate during the 1894-1986 seasons. (a) part of generation that completed development in second year; (b) one-year old; and (c) two-year old adults from first generation; (d) and (e) one- and two-year old adults from second generation; and (f) one-year old adults from third generation.

found actively boring into the stumps during the spring and summer of 1984. An inspection of the stumps in 1985 showed that the larvae completed their development in 1985. Fig:27 shows that all first instar larvae from the 1984 brood became second or older instars before the onset of the 1984/1985 winter. Some larvae without eyes (II and III instars, Table 12) developed eyes and later became last instars, pupae and emerged as adults during the 1985 season. The rest of the larvae without eyes from the 1984 brood remained unchanged until June, 1985 when they all had become fourth instars. Some of these fourth instars remained unchanged until the end of 1985 when they all became last instars and completed their development during the 1986 season. Middle instars (II, III and IV, Table 12) from the 1985 brood were collected from October, 1985 to June, 1986, indicating that these larvae would complete their development in 1986. Fig:27 shows evidence that part of the *Hylecoetus* brood completed their development in two years. The middle instars are the longest developmental stages, and this is taken to indicate the main feeding stages.

4.5.2. Development under contolled conditions

Table 25 contains data on collection of different developmental stages of *Hylecoetus* from naturally infested larch stumps and kept at 25±5°C. in 1984. Information on the development of *H. dermestoides* on agar at 20°C. is given in Tables 16 and 23 and Fig:23. Table 25 shows that the larval developmental period was reduced from 12 months (natural) to 9 months when kept at 25±5°C. However, larvae kept on agar at 20°C. nearly followed a normal course of development as illustrated in Fig:23.

Table 25. DEVELOPMENT OF *Hylecoetus* IN NATURALLY INFESTED PIECES OF WOOD OF LARCH KEPT AT 25±5°C.

Sampling Date	LARVAL INSTARS I	II&III	IV	V	PUPAE	ADULTS Female	Male
1.11.1984	0	0	7	9	0	0	0
15.11.1985	0	2	8	0	0	0	0
25.11.1985	0	0	0	1	0	0	2
4.2.1985	0	0	0	0	0	0	6
13.2.1985	0	0	0	0	0	8	5
25.3.1985	0	0	0	0	0	0	3

CHAPTER 5

CHAPTER 5

EMERGENCE AND FLIGHT PATTERNS

This section deals with investigations into the relationships between weather parameters and seasonal emergence, diurnal and seasonal flight patterns of *H. dermestoides* on the Atholl Estate, during the 1984 and 1985 seasons.

Conclusions are based on the data obtained in 1984. Owing to the poor weather in spring and summer in 1985, few adult *Hylecoetus* were actually caught in flight, the total (21) being quite insufficient to allow any conclusions to be drawn from statistical analyses.

5.1. Emergence of *Hylecoetus*

Data for seasonal emergence based on individuals recovered from caged pieces of wood cut from larch stumps and exposed roots, and flight data based on catches from different flight traps operated on the Atholl Estate, from 3 May to 30 June, 1984, are presented for *Hylecoetus* in Table 26. Emergence peaks occurred on 13 and 23 May, and 7 and 8 June, over 60 percent emerging during the month of May. No further *Hylecoetus* emerged after 19 June (Figs 28 and 29). A total of 50 adults were recovered from the cages and had a sex ratio ♀1:1♂. The relationship between emergence and flight patterns during the 1984 season is shown in Fig:30. Number of emerging adults were greatest on days when peaks of flight were recorded. Data for the emergence pattern of *Hylecoetus* in relation to time of day were not collected. However, the relationship between emergence and flight (Fig:30), seems to indicate that no appreciable delay exists between emergence and the commencement of flight. The relationship between emergence and flight was investigated further by regression analysis. Table 27 shows the recorded values for emergence, flight

and weather. The relationship between emergence and flight is linear, and has a significant correlation coefficient, r=0.6434, p=0.01, y=4.2427X+5.3574. The analysis of variance comparing emergence and flight shows that the regression of the total number of *Hylecoetus* caught in flight in a day on the daily emergence total is significant (Fig:31, Appendices 2-3).

Table 26: COMPARISON OF NUMBERS AND SEX RATIOS OF *Hylecoetus* EMERGING FROM CAGED INFESTED LARCH PIECES OF WOOD CUT FROM STUMPS, AND THOSE CAUGHT IN FLIGHT TRAPS, ON THE ATHOLL ESTATE IN 1984

Date of collection	NUMBERS EMERGING FROM CAGES				NUMBERS FROM FLIGHT TRAPS			
	Female	Male	Total	Sex ratio ♀:♂	Female	Male	Total	Sex ratio ♀:♂
May								
3	0	0	0		3	0	3	3:0
13	5	4	9	1.25:1	61	6	67	10.2:1
14	2	1	3	2:1	29	1	30	29:1
15	2	3	5	0.67:1	0	0	0	
18	1	0	1		0	0	0	
19	1	1	2	1:1	0	0	0	
20	0	1	1		0	0	0	
21	0	0	0		0	0	0	
22	0	0	0		26	2	28	13:1
23	7	2	9	3.5:1	41	5	46	8.2:1
24	1	0	1		1	1	2	1:1
25	0	1	1		0	0	0	
28	0	0	0		15	5	20	3:1
31	0	0	0		29	22	51	1.3:1
June								
1	0	1	1		0	0	0	
7	2	8	10	0.25:1	25	25	50	1:1
8	0	0	0		5	1	6	5:1
12	0	0	0		0	0	0	
13	3	1	4	3:1	1	0	1	1:0
14	0	0	0		3	0	3	3:0
15	1	1	2	1:1	15	3	18	5:1
16	0	1	1		1	0	1	1:0
17	0	0	0		0	0	0	
18					0	0	0	
20	0	0	0		3	0	3	3:0
21-30	0	0	0		0	0	0	
	25	25	50	1:1	258	71	329	3.6:1

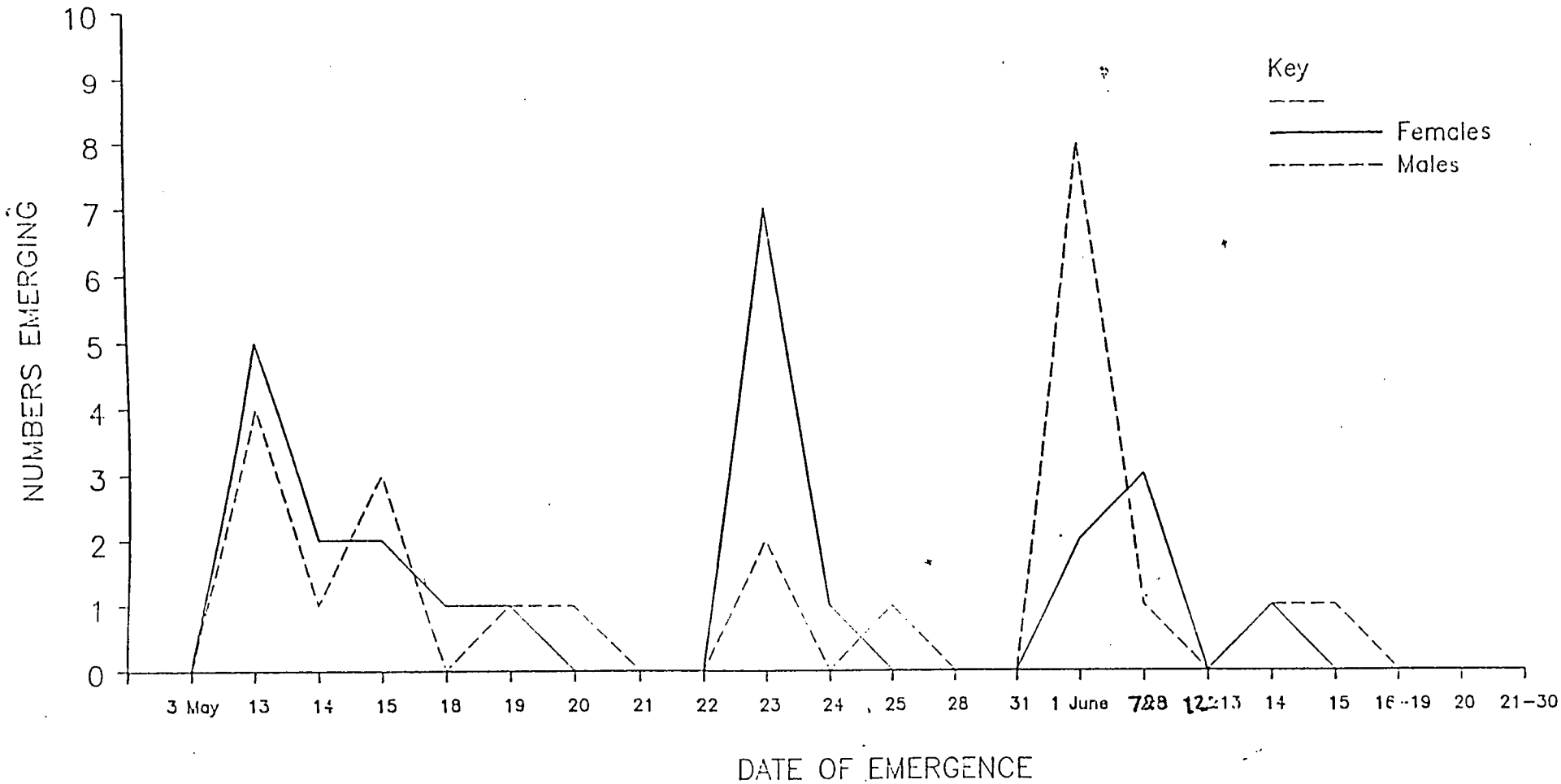


Fig: 28 Summed daily emergence totals of *Hylecoetus dermestoides* from emergence cages 2 May – 30 June, 1984.

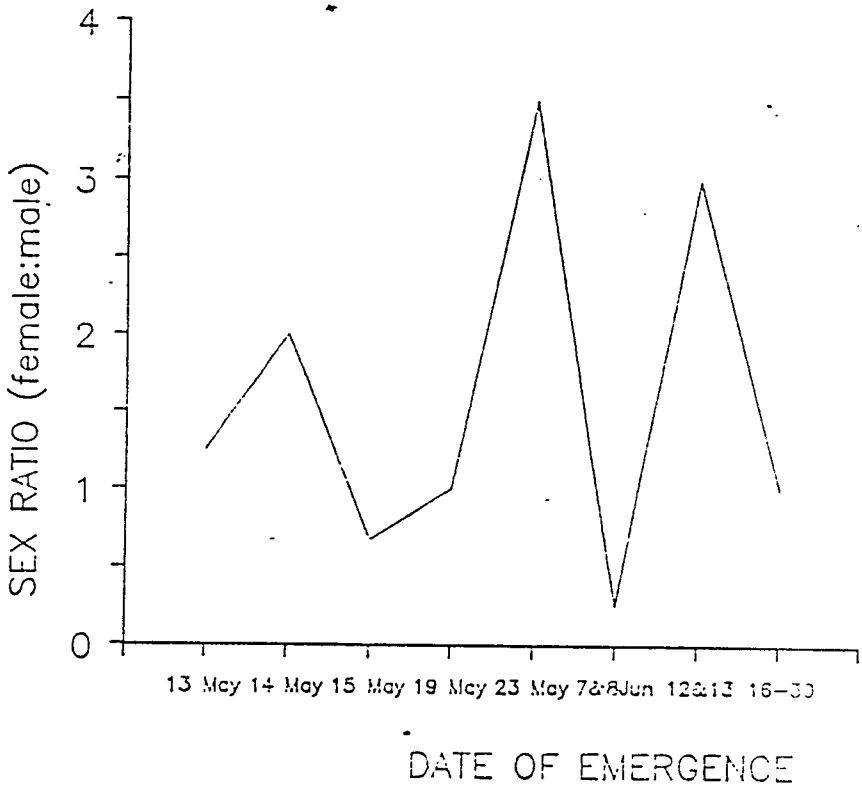


Fig:29 Relationship of female and male *Hylecoetus dermestoides* emerging from caged infested larch pieces of wood, 3 May – 30 June, 1984.

Table 27: NUMBERS OF EMERGED AND FLYING *Hylecoetus*, DAILY FLIGHT INDICES, AND WEATHER PARAMETERS FOR DUNKELD AND ARDTALNAIG, FOR 22 DAYS DURING THE 1984 SEASON

Date	NUMBERS Emerged	NUMBERS In flight	FLIGHT INDEX	DUNKELD		ARDTALNAIG				
				Max. °C.	Min. °C.	Max. °C.	Min. °C.	Grass °C.	Soil °C.	Hours of sunshine
May										
3	0	3	0.23	18	5	16.3	3.0	-1.5	10.3	7.5
13	9	67	2.91	18	1	19.5	0.5	-3.5	10.2	13.0
14	3	30	1.30	18	0	18.5	1.3	-3.0	10.5	13.1
15	5	0	0.00	18	0	14.7	5.3	1.5	11.4	2.5
18	1	0	0.00	16	3	16.5	7.0	6.7	11.0	11.6
19	2	0	0.00	15	4	16.3	3.9	0.5	11.0	6.3
20	1	8	0.35	14	5	13.2	2.0	-1.0	10.6	2.2
21	0	2	0.09	13	7	12.0	5.6	2.9	10.9	0.0
22	0	28	1.17	20	11	19.4	8.4	6.6	11.0	4.7
23	9	46	1.22	23	10	24.3	11.4	10.9	12.2	10.5
24	1	2	2.00	21	6	22.0	11.7	8.5	13.5	7.0
25	1	0	0.00	18	8	15.5	6.0	5.4	13.0	0.0
28	0	20	0.80	17	6	20.5	2.2	-3.0	11.2	8.7
31	0	51	1.96	20	8	21.5	8.0	8.0	13.0	10.4
June										
1	1	1	0.04	16	3	14.0	9.0	8.5	13.0	0.0
7	10	50	2.94	25	8	24.6	7.0	3.3	13.6	13.6
8	0	6	0.35	24	10	25.9	5.9	0.4	13.8	13.6
13	4	1	0.08	17	11	14.3	12.1	10.5	14.8	0.3
14	0	3	0.12	20	7	18.9	10.0	6.9	14.8	6.5
15	2	18	0.69	20	10	24.3	5.5	1.7	14.5	5.4
16	1	1	0.04	22	14	24.6	10.0	8.4	14.6	10.0
20	0	3	0.12	20	7	19.0	13.5	12.5	15.5	2.4

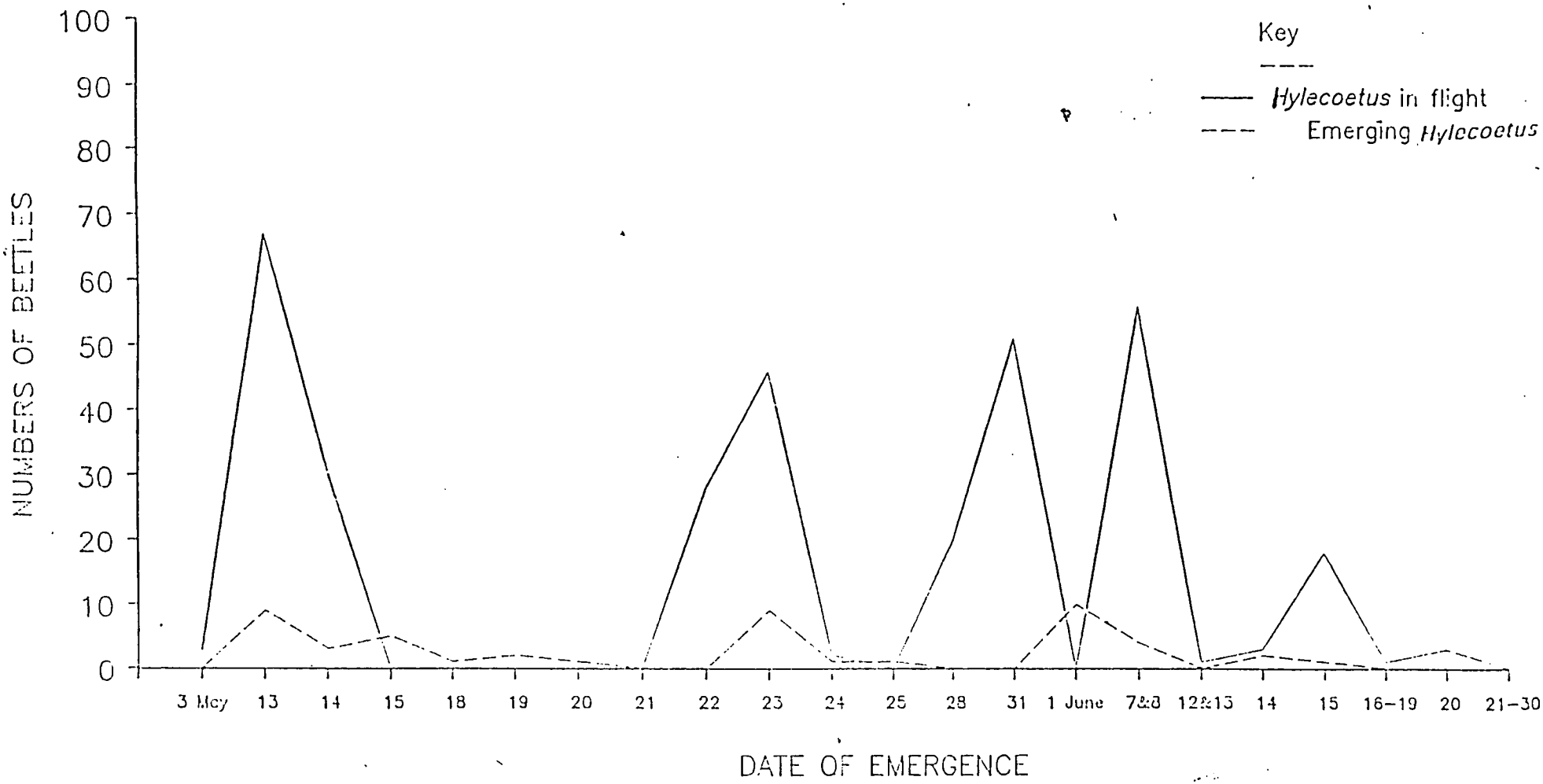


Fig: 30 Numbers of *Hylecoetus dermestoides* emerging from caged infested larch pieces of wood, and those caught in flight traps on the Atholl Estates, 2 May – 30 June, 1984.

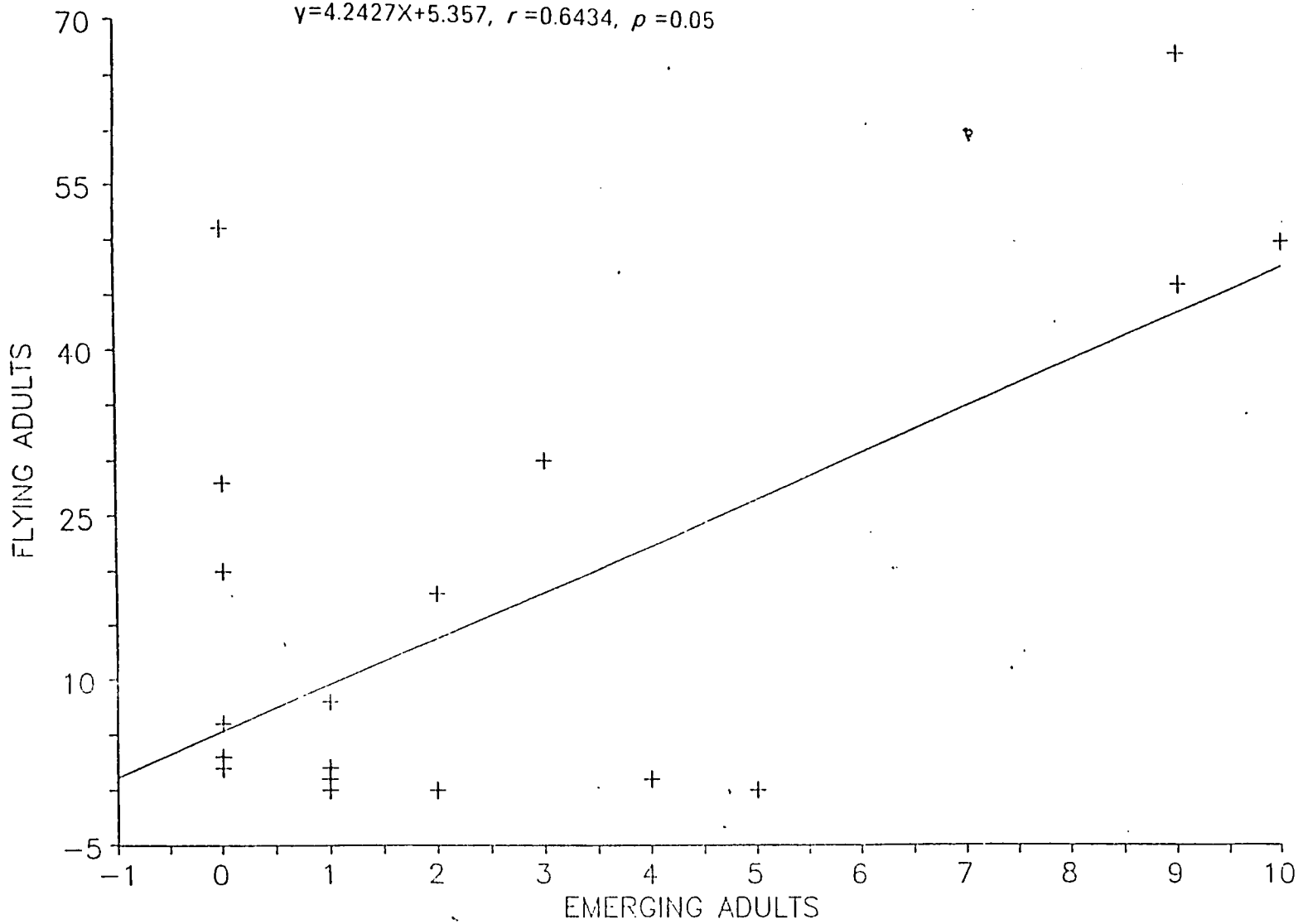


Fig:31 Relationships between numbers of flying and emerging *Hylecoetus dermestoides* on the Atholl Estate during the 1984 season.

5.1.1. Influence of weather parameters on emergence of *Hylecoetus*

5.1.1.1. Light

Emerging *Hylecoetus* were collected from 4 diet cages, each cage facing one of the cardinal points, i.e., north, west, south and east respectively. The relationship between the position of each cage (which was related to the direction of the sun), and numbers emerging into the cage, was investigated. Fig:7 (page 58) shows that cages 3 and 4 were facing the direction of the sun, while cages 1 and 2, were facing away from the direction of the sun respectively. Table 28 shows numbers emerging from the four cages. Cage 4 (18) and cage 1 (16), had higher numbers than cage 2 (7) and cage 3 (8) respectively.

Table 28: NUMBERS OF *Hylecoetus* RECOVERED FROM 4 DIET CAGES ATTACHED TO A LARGE (60x60x60cm) PLYWOOD BOX ON THE ATHOLL ESTATE DURING THE 1984 SEASON

Date of collection	Cage 1 Facing North	Cage 2 Facing West	Cage 3 Facing South	Cage 4 Facing East	Total
May					
13	1	0	2	6	9
14	0	0	1	2	3
15	2	0	1	2	5
18	0	0	0	1	1
19	1	0	0	1	2
20	0	0	0	1	1
23	6	2	0	1	9
24	1	0	1	0	1
25	0	0	1	0	1
June					
1	0	0	1	0	1
7&8	3	3	0	4	10
12&13	2	2	0	0	4
15	0	0	2	0	2
16-19	1	0	0	0	1
Total	16	7	8	18	50

5.1.1.2. Air temperature, soil temperature and sunshine

Due to the limitation in the number of weather parameters that were measured at Dunkeld in 1984, it was thought reasonable encugh to obtain additional weather information from Ardtalnaig being the nearest permanent meteorological station. At Dunkeld data were obtained for maximum and minimum air temperatures, rain fall, wet and dry bulb temperatures and relative wind speed, whereas at Ardtalnaig data for the following weather variables were obtained: (1) maximum and minimum air temperatures; (2) minimum grass and soil temperatures; (3) relative wind speed; and (4) hours of sunshine. The influence of all weather parameters recorded at Dunkeld and Ardtalnaig respectively, on the numbers of *Hylecoetus* emerging was studied using regression analysis. The mean maximum temperatures were 18.8° and 18.9°, minimum temperatures were 6.5° and 6.8°C. for Dunkeld and Ardtalnaig. None of the weather parameters were significant predictors of numbers of emerging *Hylecoetus*.

5.2. Flight activity of *Hylecoetus*

Previous studies show that weather parameters influence the flight activity of wood-boring Coleoptera. These studies show that each weather variable, either taken singly or in association with one or more weather variables, will influence insect behaviour in a way that will depend upon the species under investigation. Due to the non-availability of quantitative data on the relationship between *Hylecoetus* and weather, both data on *Hylecoetus* diurnal and seasonal flight and weather were obtained and their relationships examined. Weather variables considered are: (1) maximum air temperature; (2) minimum air temperature; (3) minimum grass temperature; (4) soil temperature at the 10 cm depth; (5) percent relative humidity; (6) rainfall; (7) relative wind speed; and (8) hours of sunshine.

5.2.1. Diurnal flight pattern

The diurnal flight was established using numbers of *Hylecoetus* caught in flight at different trapping sites, during the 1984 season. Trapping of flying individuals was carried out in areas listed in Table 8, at an elevation of 100–330 m. (Maps 2 and 3, page 56), with sufficient naturally infested stumps to provide an adequate population. The diurnal flight of *Hylecoetus* is a study of the relationships between numbers of *Hylecoetus* in flight throughout the day and weather variables. Hourly collections of adults were made between 10.00 and 20.00HRS at attractive larch stumps, standing dead trees and ethanol-baited flight traps. Hourly readings of weather variables were made during the same periods, and numbers of adults on the wing were correlated with the weather variables.

5.2.1.1. Weather variables and diurnal flight

Numbers of adults caught in flight together with weather parameters (Table 29) were transcribed into graphs (Figs 32–35). Due to differences between days in numbers of traps operated, the total daily catch was divided by the number of traps operated on that day. This gives an indication of relative adult activity, for the different days, and is referred to as the "flight index". Temperature, relative humidity and wind speed are presented as means for each day for the period 10.00 to 20.00HRS. Number of hours of sunshine for each flight day are given.

Before analysis of data, percent relative humidity was subjected to arcsine transformation (page 82). Table 29 shows untransformed values for % relative humidity. Regression analysis was employed to study the relationship between daily activity of *H. dermestoides* and the meteorological parameters. Using all the measured weather parameters, it was found that when the flight index is regressed on individual independent variables, temperature, % relative humidity and hours of sunshine gave significant predictions of activity of

Hylecoetus. The relationship between flight index and relative wind speed has a negative and non-significant correlation coefficient. The best of the independent variables, hours of sunshine, does not seem to give a completely satisfactory description of catch index, because, although sunshine has the largest correlation coefficient (r), temperature and % relative humidity have equally significant values of r. The selection of the best predictor variable was continued by taking each possible pair of independent variables to determine which pair would give the best prediction of *Hylecoetus* activity. Five of the six possible pairs of independent variables had significant correlation coefficients, and only the temperature with relative wind speed pair had a non-significant correlation coefficient. Judging by the magnitudes of the correlation coefficients of the regressions of catch index on pairs of independent variables, no single pair of weather variables seemed exceptionally the best (Appendix 4). However, % relative humidity with wind speed had the largest r value. The selection process was continued by analysing the possible triples (Appendix 5). All triples had significant correlation coefficients (Appendices 6 and 7). Temperature, % relative humidity and wind speed, had the highest r value. The regression of flight index on all four weather parameters gave the best prediction (Appendix 8).

Regressions of catch index on different combinations of weather parameters including those sets of independent variables containing the variable wind speed produced significant correlations (Appendices 4, 5, 6 and 7). A summary of information on the regressions of catch index of *Hylecoetus* on different sets of independent weather parameters is presented in Appendix 9.

Table 29: MEAN NUMBERS OF *Hylecoetus* CAUGHT IN FLIGHT AND MEANS FOR WEATHER PARAMETERS FOR THE FLIGHT PERIODS ON THE ATHOLL ESTATE DURING THE 1984 SEASON

Date	Total catch	Traps operated	Daily catch index	METEOROLOGICAL PARAMETERS Temp.(°C.)	%relative humidity	Wind velocity (m/s)	Hours of sunshine
May							
13	51	18	2.83	17.45	36.85	2.182	13.0
14	29	18	1.61	15.77	37.04	2.515	13.0
17	0	18	0.00	14.61	46.65	3.896	11.6
18	0	18	0.00	14.61	46.65	3.896	11.6
23	45	18	2.50	22.11	40.78	1.954	10.5
24	2	18	0.11	15.55	74.96	1.662	7.0
28	20	25	0.80	15.39	56.72	1.659	8.7
29	0	25	0.00	15.21	61.62	1.931	7.6
31	21	25	0.84	16.80	65.45	1.548	10.4
June							
7	36	17	2.11	22.86	52.19	1.824	13.6
20	1	12	0.08	19.20	58.67	3.036	2.4

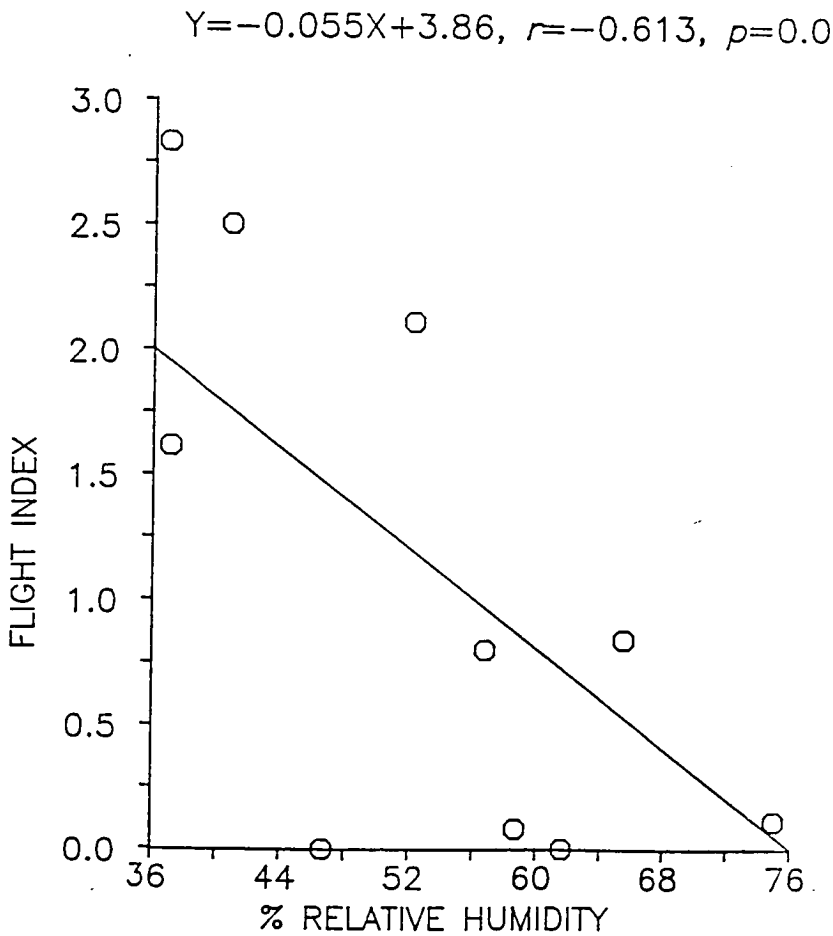


Fig:32 Relationships between flight index of *H. dermestoides* and relative humidity on the Atholl Estate during the 1984 season.

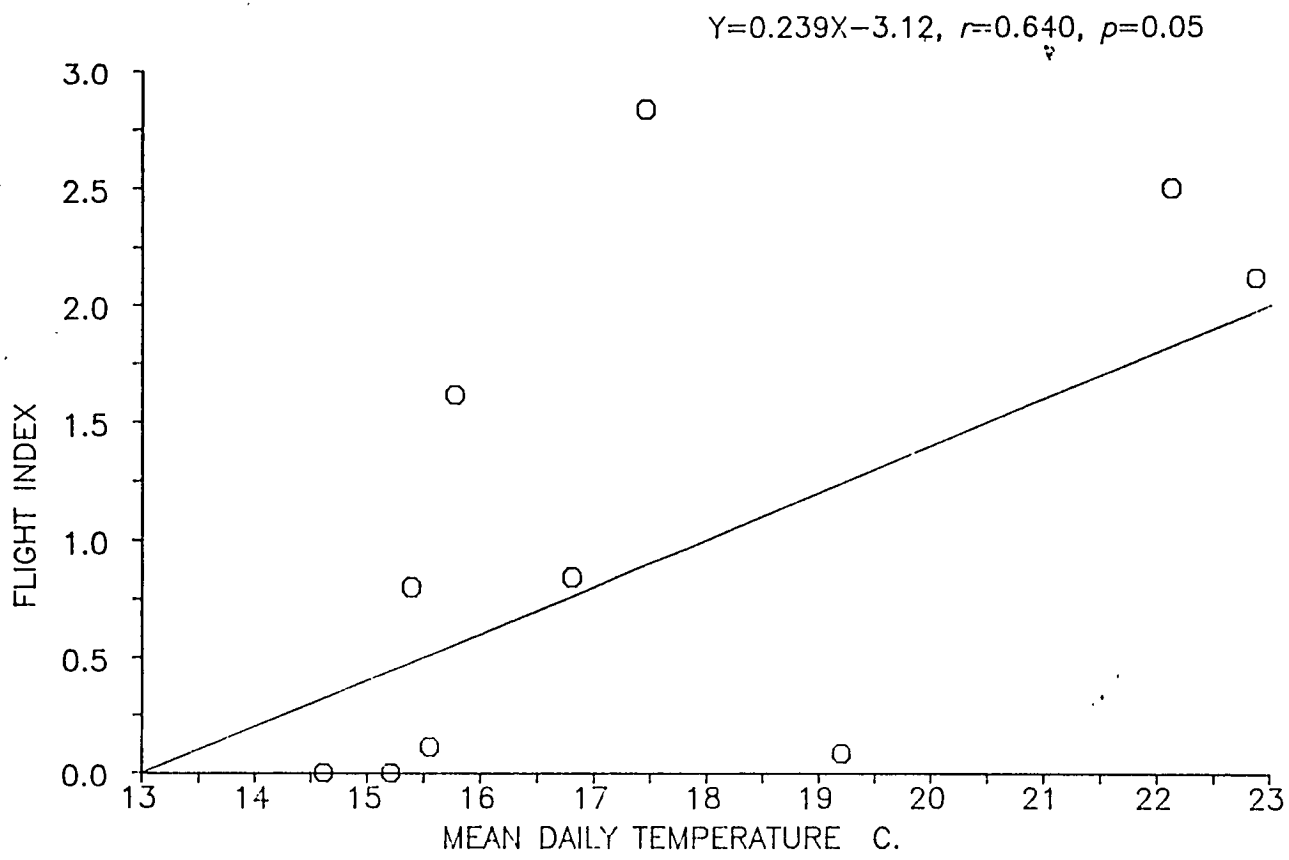


Fig:33 Relationships between catch index of *Hylecoetus dermestoides* and mean daily temperature on the Atholl Estate during the 1984 season.

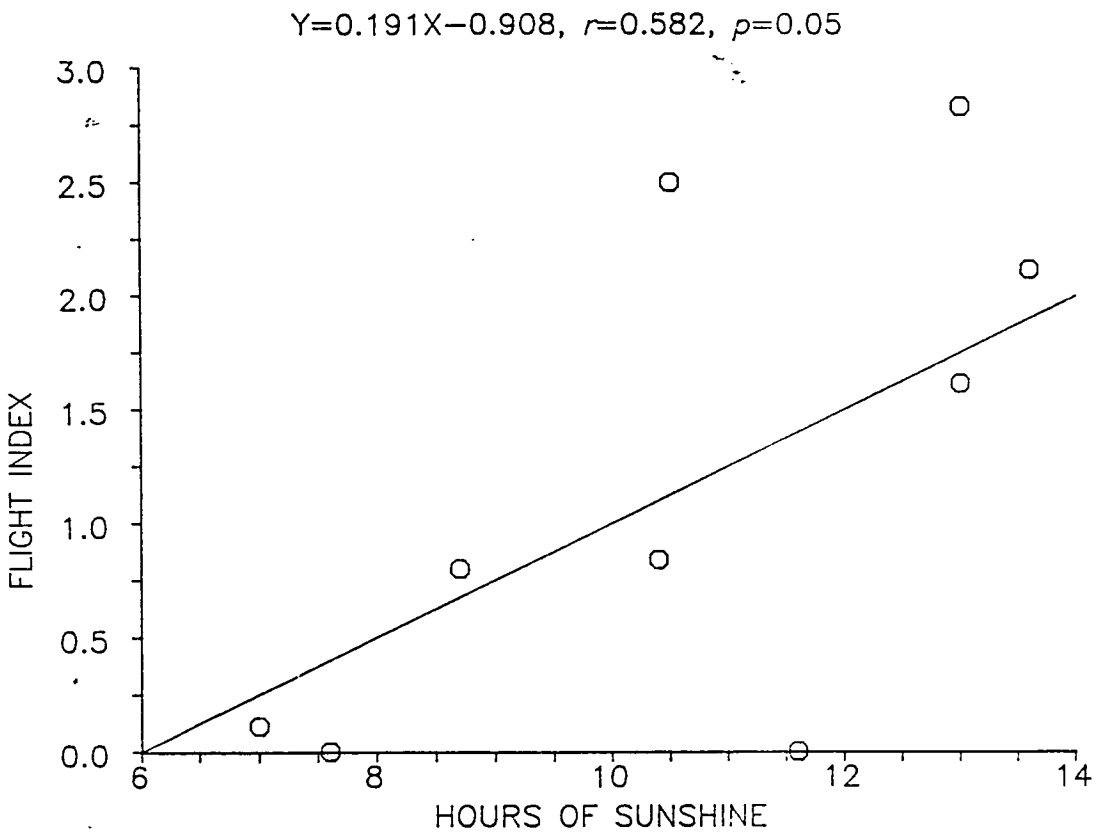


Fig:34 Relationships between catch index of *Hylecoetus dermestoides* and hours of sunshine on the Atholl Estate in 1984.

5.2.1.2. Further analysis of the influence of temperature on *Hylecoetus* flight

A further evaluation of the influence of temperature on the daily flight of *Hylecoetus* was carried out. Since temperature is an important weather variable influencing *Hylecoetus* activity, and since *Hylecoetus* activity began after temperature reached a threshold necessary for flight, the relationship between *Hylecoetus* activity and temperature below and above the threshold necessary for flight was investigated in greater detail. Other workers (Bakke, 1968; McLean and Borden, 1979) have shown that the period during which temperature exceeds the threshold for flight, and the temperature range (in degrees) above the threshold for flight are important weather variables influencing the activity of wood-destroying Coleoptera. Therefore, the relationship between *Hylecoetus* activity and the number of hours during which temperature exceeded the threshold for flight, and the temperature range above the threshold was examined. Numbers of catches and weather data for 15 days with *Hylecoetus* were examined (Table 30). A temperature of 15°C. was regarded as the temperature threshold necessary for adult *Hylecoetus* activity (Fig:33).

Figs 35 and 36-37 show the relationships between catch index and maximum temperature and other temperature factors respectively. *Hylecoetus* flight is significantly correlated with both the number of hours the temperature exceeded the 15°C. threshold and the range of temperature above the threshold.

Hylecoetus flight is more influenced by the range of temperature above the 15°C. threshold than the duration (hours) the temperature exceeded the threshold.

Table 30 *Hylecoetus dermestoides* FLIGHT AND WEATHER DATA FOR 15 DAYS ON THE ATHOLL ESTATE IN 1984

Date	Total daily catch	Daily index	Max. temp.(°C.) (°C.)	Hrs with temp. >15°C.	Degree(°C.) above 15°C.
April					
26	0	0.00	24.5	11.0	43.5
May					
6	0	0.00	12.4	0.0	0.0
7	0	0.00	11.1	0.0	0.0
9	0	0.00	13.3	0.0	0.0
10	0	0.00	13.2	0.0	0.0
13	67	2.91	19.3	9.0	24.5
14	30	1.30	18.5	8.0	12.0
17	0	0.00	15.0	0.0	0.0
18	0	0.00	16.5	5.0	3.5
23	46	2.00	24.3	11.0	73.0
28	20	0.80	20.5	6.0	8.5
29	0	0.00	19.1	3.0	3.5
31	51	1.96	15.9	8.0	19.0
June					
7	50	2.94	24.6	11.0	78.0
20	3	0.11	19.0	10.0	10.0

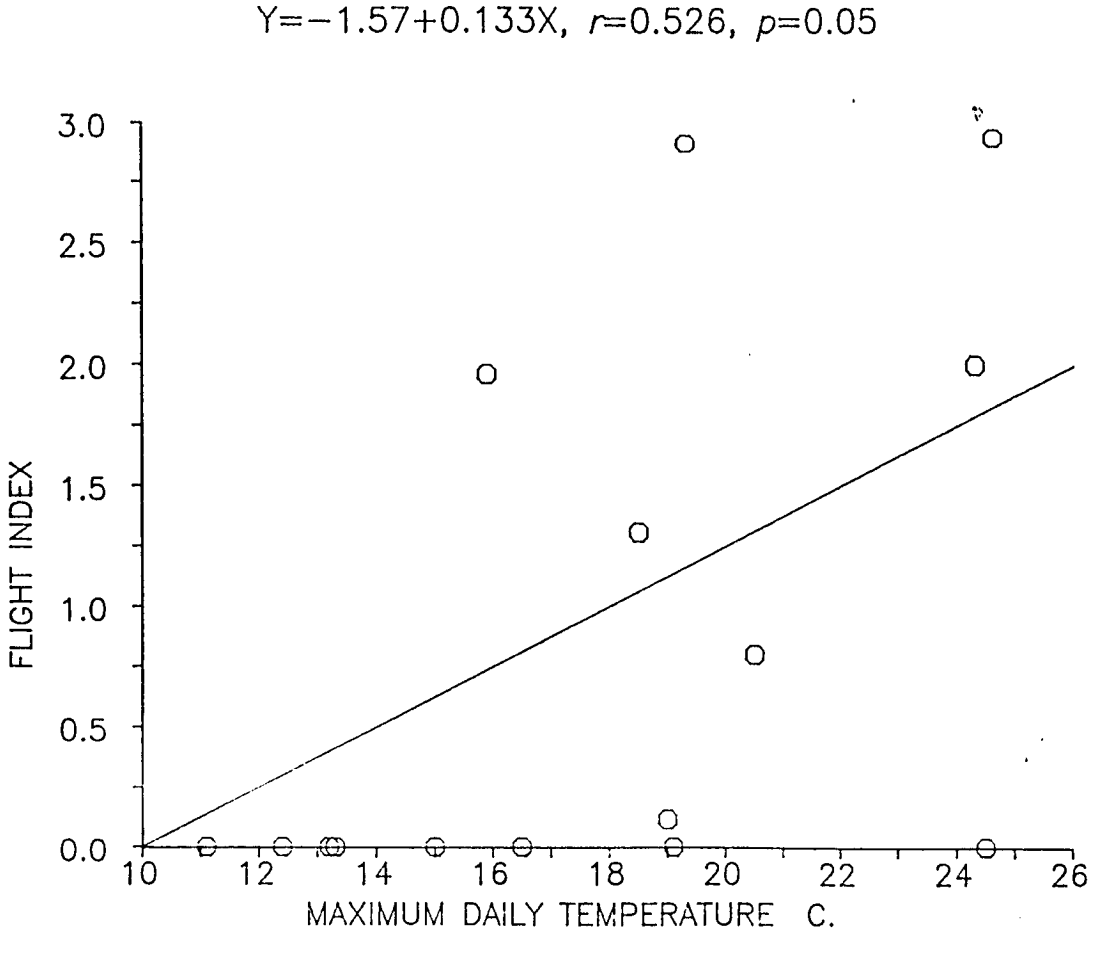


Fig:35 Relationships between catch index of *Hylecoetus dermestoides* and maximum daily temperature on the Atholl Estate in 1984.

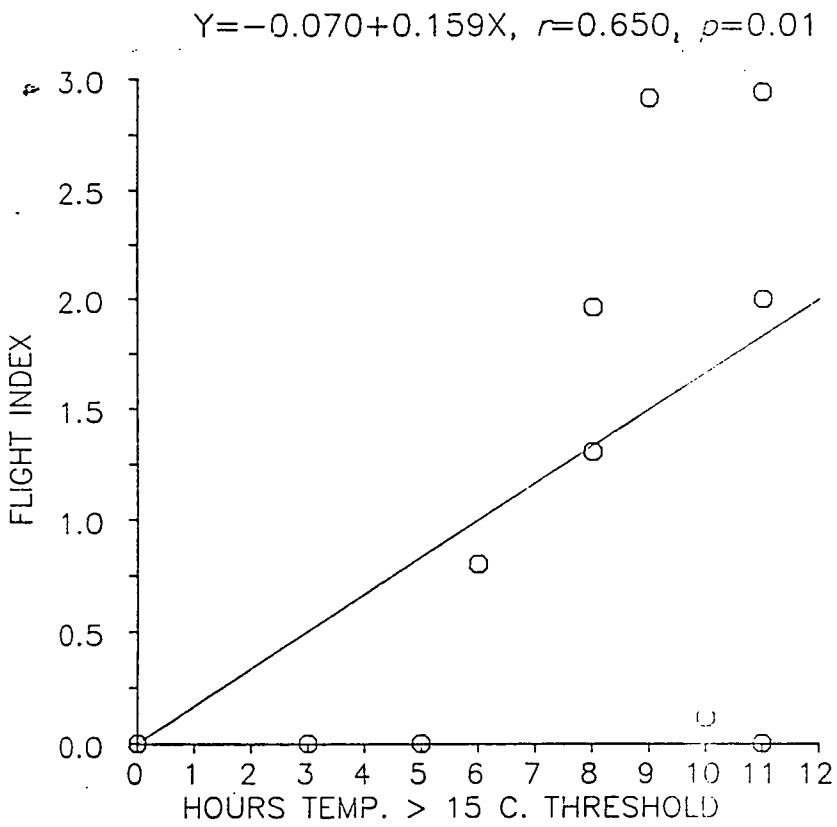


Fig:36 Relationships between catch index of *Hylecoetus dermestoides* and hours with temperature > 15 C. threshold on the Atholl Estate in 1984.

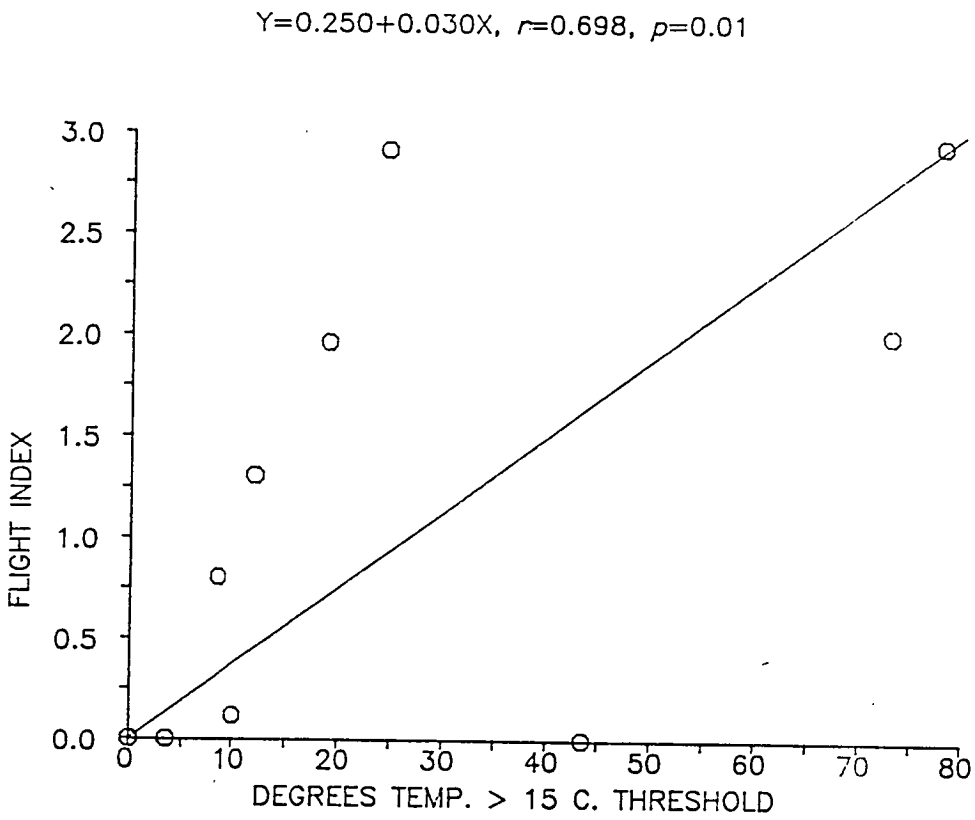


Fig:37 Relationships between catch index of *Hylecoetus dermestoides* and temperature above the 15 C. threshold on the Atholl Estate in 1984.

5.2.2. Flight pattern during the day

This section deals with *Hylecoetus* flight at hourly intervals on certain flight days and weather parameters influencing flight. Flight data obtained were based on numbers of adults landing at attractive standing dead larches. Adults were hand picked and weather variables were recorded at hourly intervals during certain flight days during the 1984 season. Table 31 shows information on the response of *Hylecoetus* to standing dead larches related to time of day. There was a peak of activity around 13.00 to 15.00, and less activity in the morning and evening. Males and females behaved similarly in this respect.

5.2.3. Flight of *Hylecoetus* during the day in relation to weather conditions

The influence of weather on the diurnal flight pattern of *Hylecoetus* was investigated using regression analysis. In order to examine the relationship of the catches throughout the day with the meteorological variables (temperature, wind speed, and percent relative humidity) the meteorological parameters were summed on an hourly basis and meaned. Fig:38 shows that the relationship between catches and the weather variables and time of day.

5.2.4. Comparison of weather parameters on "flight and non flight days"

Linssen (1959) considered *H. dermestoides* to be an evening flyer, therefore, one might expect *a priori* that lower evening temperatures would be the most closely correlated with *Hylecoetus* flight. Southwood (1960) indicates that the maximum air temperature usually occurs in the early afternoon and the minimum temperature just before dawn. In this study, *Hylecoetus* was found to be a day flyer with peak flight occurring in the afternoon (Fig:38). Therefore, higher *Hylecoetus* catches would be expected on days with higher maximum temperatures than those days with lower maximum temperatures. Soil and minimum air temperatures are not significantly correlated with the daily *Hylecoetus* catch, expressed as the catch index. *Hylecoetus* flight may be correlated with rainfall. At the beginning of the flight period during 1984

Table 3.1: LANDING BY *Hylecoetus dermestoides* ON STANDING DEAD LARCHES RELATED TO TIME OF DAY

Date	10.00 ♀	10.00 ♂	11.00 ♀	11.00 ♂	12.00 ♀	12.00 ♂	10.00-12.00HRS ♀	10.00-12.00HRS ♂	10.00-12.00HRS ∑	13.00 ♀	13.00 ♂	14.00 ♀	14.00 ♂	15.00 ♀	15.00 ♂	13.00-15.00HRS ♀	13.00-15.00HRS ♂	13.00-15.00HRS ∑	16.00 ♀	16.00 ♂	17.00 ♀	17.00 ♂	18.00 ♀	18.00 ♂	19.00 ♀	19.00 ♂	20.00 ♀	20.00 ♂	16.00-20.00HRS ♀	16.00-20.00HRS ♂	16.00-20.00HRS ∑
May																															
13	0	0	0	0	2	0	2	0	2	3	3	2	0	14	3	19	6	25	2	0	1	0	0	0	0	0	0	0	3	0	3
14	0	0	6	0	0	0	6	0	6	2	0	5	0	1	0	7	1	8	8	1	5	0	0	0	2	0	0	0	15	1	16
23	0	0	0	0	1	0	1	0	1	11	0	10	2	5	1	26	3	29	3	0	0	0	9	1	2	1	0	0	14	2	16
28	0	0	0	0	0	0	0	0	0	0	0	6	2	7	3	13	5	18	1	0	1	0	0	0	0	0	0	0	2	0	2
31	0	0	4	1	0	0	4	1	5	2	2	7	3	4	0	13	5	18	2	0	0	0	0	0	0	0	0	0	2	0	2
June																															
7	0	0	0	0	0	0	0	0	0	7	7	3	7	7	7	17	21	38	2	2	6	2	0	0	0	0	0	0	8	4	12
Sub-Total	0	0	10	1	3	0	13	1	14	25	12	33	14	38	14	95	41	136	18	3	13	2	9	1	4	1	0	0	44	7	51
Grand Total	0		11		3					37		47		52					21		15		10		5		0				

(25 April) the daily maximum temperature was 23.2°C. with 12 hours of sunshine. The seasonal maximum (25.9°C.) occurred on 8 June (13.6 hours of sunshine) and at the last collection date (20 June, 1984) the maximum temperature was 19.0 °C. and there were 2.4 hours of sunshine respectively. Few *Hylecoetus* flew before the end of April, 1984. After the end of April, the catch index increased and the first seasonal peak occurred on 13 May (maximum temperature 19.3°C.; and 13 hours of sunshine). The highest daily catch index occurred on 7 June (maximum temperature 24.6°C.; 13.6 hours of sunshine).

The finding that flight of *Hylecoetus* may be sufficiently correlated with weather parameters (Table 32) was investigated further, by carrying out calculations of correlation and regression coefficients on different sets of data.

5.2.4.1. Possible triggering effect of weather parameters on *Hylecoetus* flight

Daily flight indices were regressed on the weather variables for the same day; one; two; three; four days and so on up to seven days prior to the flight period. Only maximum temperature and hours of sunshine recorded on the flight day showed significant regression and correlation coefficients. The regression of *Hylecoetus* flight on the maximum temperature had the highest correlation coefficient (r=0.3927, p=0.01, y=0.0629X-0.8712). This indicates that maximum temperature may be the most important correlate with daily flight activity. Number of hours of sunshine were the second important correlate with *Hylecoetus* flight (r=0.3420, p=0.01, y=0.0450X-0.0661). The relationships of *Hylecoetus* flight and other weather parameters had negative and non-significant correlations.

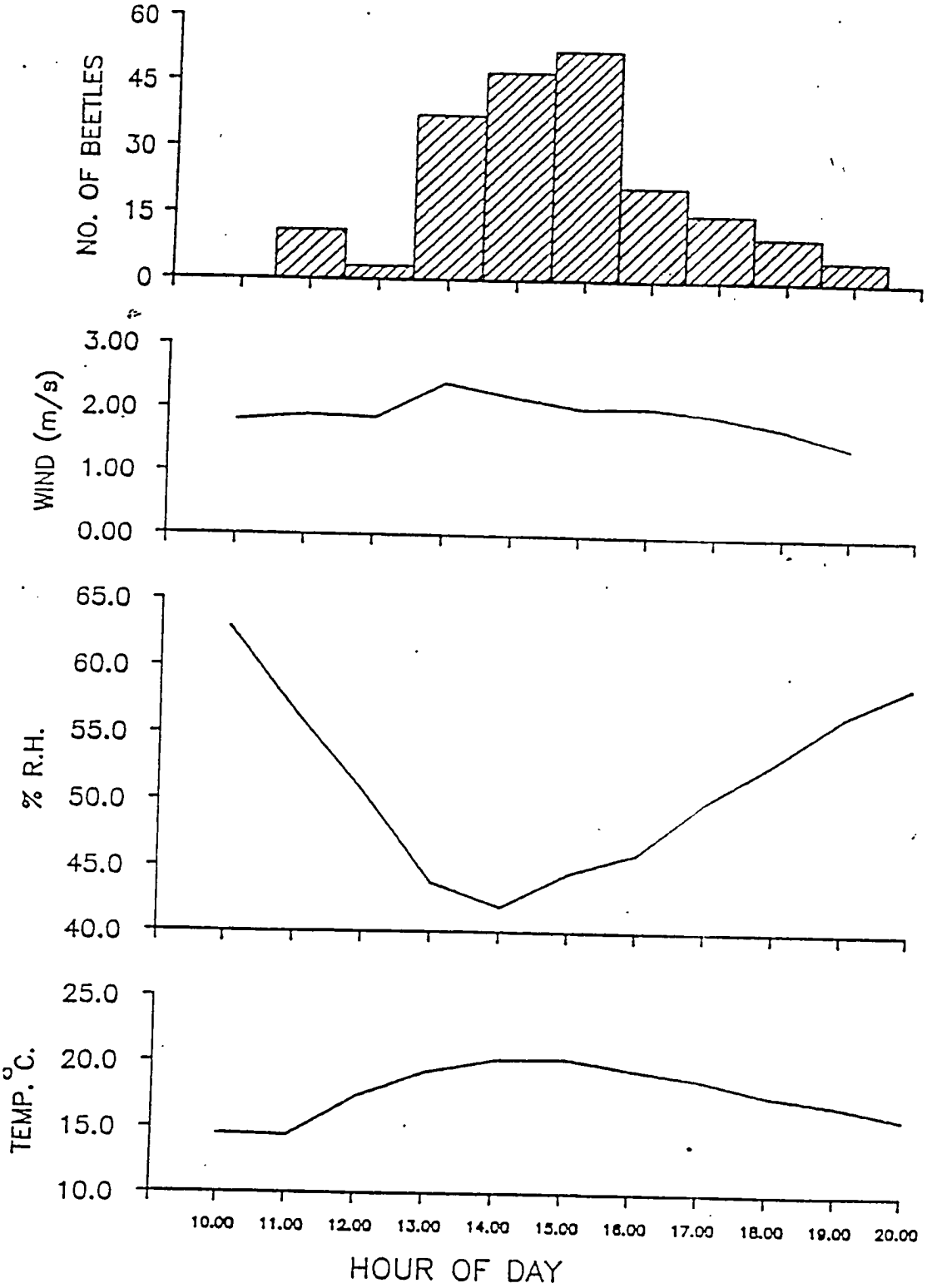


Fig: 38 Visitation rates of *Hylecoetus dermestoides* beetles at standing dead (or dying) larch trees based on 1984 flight records.

Table 32: COMPARISON OF CLIMATIC FACTORS ON DAYS "WITH" AND DAYS "WITHOUT" *Hylecoetus* FLIGHT BETWEEN 18 APRIL AND 30 JUNE ON THE ATHOLL ESTATE DURING THE 1984 SEASON.

	DAYS WITH FLIGHT (n=21)		DAYS WITHOUT FLIGHT (n=53)
Highest max. temp.(°C.)	25.90		25.00
Lowest max.temp.(°C.)	12.00		10.20
Mean max. (°C.)	19.70		16.24
Standard deviation	3.9665		3.2152
T-Value		3.903	
p =		0.001	
Max. hrs of sunshine	13.60		13.00
Min. hrs	0.00		0.00
Mean hrs	7.967		5.572
SD	4.0115		4.64
T-Value		2.08	
p =		0.05	
Highest min. temp.(°C.)	13.50		15.00
Lowest minimum	0.50		-0.40
Mean min. (°C.)	6.36		6.4887
SD	3.838		
T-Value		0.13005	
p =		n.s.	
Max. min. grass temp.(°C.)	12.50		13.50
Lowest min.	-3.50		-5.10
Mean	3.36		3.81
SD	5.087		4.82
T-Value		0.3572	
p =		n.s.	
Highest wind speed (m/s)	4.6399		3.7836
Lowest (m/s)	0.7868		0.5042
Mean (m/s)	1.9191		1.9748
SD	0.829		0.868
T-Value		0.2520	
p =		n.s.	
Highest rainfall (mm)	8.50		19.100
Lowest (mm)	0.00		0.000
Mean (mm)	0.871		1.674
SD	1.922		2.815
T-Value		1.1997	
p=		n.s.	
Highest %R.H.	77.00		83.20
Lowest	48.80		48.20
Mean	61.62		62.42
SD	7.107		8.401
T-Value		0.045	
p =		n.s.	
Highest min. soil temp.(°C.)	15.50		17.00
Lowest	9.70		7.50
Mean	12.195		12.364
SD	1.869		2.727
p =		n.s.	

Table 33: FLIGHT ACTIVITY OF *Hylecoetus dermestoides* ON THE ATHOLL ESTATE DURING THE 1984 SEASON.

APRIL

it ie used	Trap no.	18	19	20	21	22	23	24	25	26	27	28	29	30
ch logs	1													
ndow	2								2					
ht	3													
	4													
os	A												1	1
ch stumps	B													
oss vane	C													
os)	D													
	E													
	F													
anding	DT-1													
ad larch	DT-2													
ees	DT-3													
	DT-4													
	DT-5													
	DT-6													
	DT-7													
	DT-8													
	DT-9													
	DT-10													
	DT-11													
	DT-12													
ndow trap h ethanol														
T[1]	1(1*)													
T[2]	2(0)													
T[3]	3(2)													
T	4(0)													
T	5(2)													
T	6(1)													
T	7(2)													
T	8(1)													
T	9(0)													
ss	MFT													
ntrol	MFT													
ss	MFT													
ntrol	MFT													
al catch									2				1	1
nber of	traps								7				13	13
ly catch	index								0.29				0.08	0.08

MAY

it ie used	Trap no.	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
ch logs	1		1																													
ndow	2																															
ht	3			1										4																		
	4																															
os	A		2	2										5																		
ch stumps	B													4																		
oss vane	C														1																	
os)	D		1											1																		
	E													2																		
	F																															
anding	DT-1													14							2		1	5								
ad larch	DT-2													13	6						3		9	14					5			3
ees	DT-3													4	5						2		4	6								
	DT-4													1	2							1	4	3								
	DT-5													2	6								1	9					4			
	DT-6														3								3	4								
	DT-7													17	7						1	1	6	5								
	DT-8																															
	DT-9																															
	DT-10																															
	DT-11																															
	DT-12																								2				11			18
ndow trap h ethanol																																
T[1]	1(1*)																															17
T[2]	2(0)																															9
T[3]	3(2)																															
T	4(0)																															
T	5(2)																															
T	6(1)																															
T	7(2)																															
T	8(1)																															
T	9(0)																															
ss	MFT																															
ntrol	MFT																															
ss	MFT																															3
ntrol	MFT																															1
al catch			4	3										67	30						8	2	2	46	2				20			51
nber of	traps		13	13										23	23						23	23	23	23	23				25			26
ly catch	index		0.31	0.23										2.91	1.30						0.35	0.09	1.21	2.00	0.07				0.80			1.96

JUNE

it ie used	Trap no.	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20
ch logs	1																				
ndow	2																				
ht	3																				
	4																				
os	A																				
ch stumps	B																				
oss vane	C																				
os)	D																				
	E																				
	F																				
anding	DT-1																				
ad larch	DT-2							17								3					
ees	DT-3							1													1
	DT-4																				
	DT-5																				
	DT-6																				
	DT-7																				
	DT-8																				
	DT-9																				
	DT-10																				
	DT-11																				
	DT-12							18	2					2	2	2					
ndow trap h ethanol																					
T[1]	1(1*)							9	2							4	1				2
T[2]	2(0)																				
T[3]	3(2)																				
T	4(0)																				
T	5(2)																				
T	6(1)														1	2					
T	7(2)															7					
T	8(1)																				
T	9(0)																				
ss	MFT							2													
ntrol	MFT																				
ss	MFT							2	1												
ntrol	MFT							1	1												
al catch								50	6					2	3	18	1				3
nber of	traps							17	17					25	25	26	26				26
ly catch	index							2.94	0.35					0.04	0.12	0.69	0.04				0.12

WBT[1]=window barrier traps (Chapman and Kinghorn 1958, Bakke, 1968); MFT[2]=multiple funnel traps (Lindgren, 1983);

CTV[3]=cross vane traps (Bakke, 1975,1977,1978; Botterweg, 1982); numbers in parentheses (1*)=number of ethanol dispensers used per trap.

CHAPTER 6

CHAPTER 6

HOST SELECTION-PRIMARY ATTRACTION

6.1. Response of *Hylecoetus* to larch logs, stumps and standing dead trees

6.1.1. Flight elevation

In late April, 1984 *H. dermestoides* were observed flying to stumps and standing dead trees of European larch on the Atholl Estate. Generally *Hylecoetus* were observed flying at about 2-3 m. above the ground. When approaching the host material the flight was rather firmly directed and *Hylecoetus* were observed to lose height. Turns to the left or right of the straight line of approach were frequent and were usually about 1-2 m., and *Hylecoetus* turned back quickly until the hosts were reached. This approach has been described as being klino-kinetic along the gradient of concentration of the attractant and this behaviour pattern was observed in *Trypodendron lineatum* by Rudinsky and Daterman (1964). When *Hylecoetus* flew past the hosts, at about 1-2 m from the host a return flight was made towards the host and flew past the host at a distance of about 1-2 m, then turned and flew towards the host again while making left to right turns until successful landings were made (Fig:39). Landing zones on the trees were in the region between the bases of the trees up to about 1 m. along the boles of the trees. However, most *Hylecoetus* landed or moved down to the bases of the trees in a zone about 30 cm. high.

6.1.2. Influence of wind and light on flight of *Hylecoetus*

The mode of navigation by *Hylecoetus* was observed frequently on the approach to the larch stumps and standing dead trees. These field observations showed that *Hylecoetus* flew head to wind when approaching the hosts. On approaching the standing trees, *Hylecoetus* which would be initially flying with

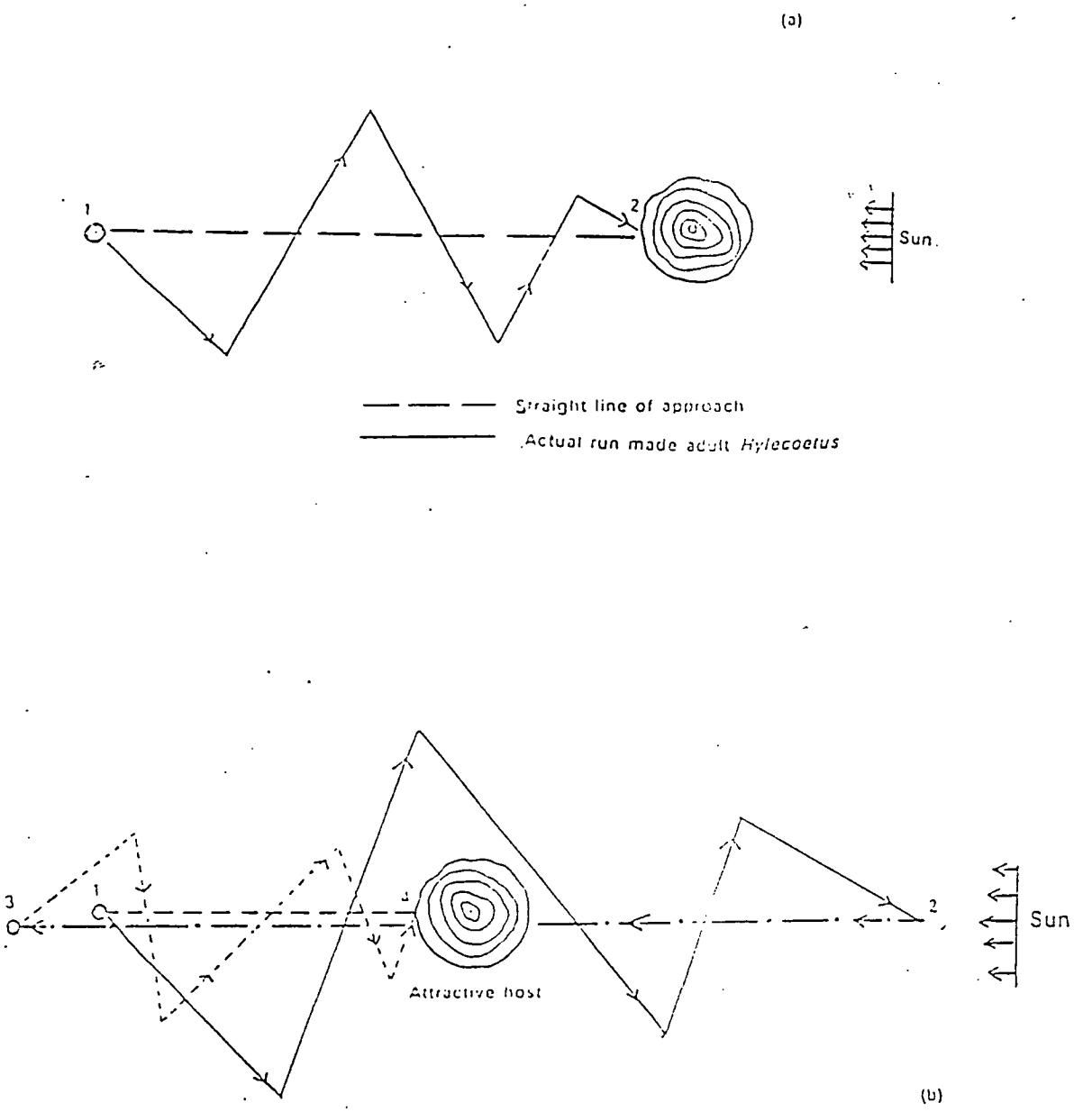


Fig: 39 Runs of adult *Hylecoetus* to attractive Larch host: (a) first attempt of flight toward host results in successful landing; (b) adult flies past the host. (1–2) then makes a return flight in an almost straight line (2–3), then turns to make second and successful landing on host (3–4).

the wind, changed course and flew head to wind towards the hosts. *Hylecoetus* landed on host trees or stumps in zones sheltered from the prevailing wind and the sun. During the flight periods the sun was generally in the south-west direction in relation to, for example, standing dead trees, and the prevailing winds generally blew from the south-west. Therefore, most *Hylecoetus* landed on the north-west aspects of the host stumps or trees. No flight was recorded during periods in which temperatures greater than 15°C. were accompanied by generally strong winds from the north-west. Such winds were generally associated with periods of rain.

The relationship between *Hylecoetus* and light was investigated further by using adult *Hylecoetus* from naturally infested stumps. Adult *Hylecoetus* were collected and kept alive in glass jars lined with paper. *Hylecoetus* were later released on a table in a room with a 60W electric light bulb. All *Hylecoetus* released flew towards the light source. Before take-off all *Hylecoetus* faced the source of light, and flew below the light source at a height of about 2 m. *Hylecoetus* made several rapid circular flights around the light bulb, and these movements were roughly in form a figure of eight (8), with the centre of the 8 being the source of light. Most *Hylecoetus* were in flight for 2-3 minutes before landing. *Hylecoetus* did not fly during the second night.

6.1.3. Behaviour of *Hylecoetus* upon landing on hosts

Many adult *Hylecoetus* were observed while landing on stumps and standing dead trees of larch. After landing on the host, *Hylecoetus* made rapid movements over the bark surface in a searching manner. *Hylecoetus* occasionally stopped moving and while resting, females extruded their ovipositors and inserted them in crevices in the bark or in the foliose lichen on the host. When not active, *Hylecoetus* generally settled in zones sheltered from the wind. While male *Hylecoetus* were on the hosts they expanded their

maxillary palpi and then began making rapid movements on the bark surface. The expanded maxillary palpi of male *Hylecoetus* produced a blowing or cooling effect on the back of the hand. The cooling effect stopped when the maxillary palpi contracted. Generally male *Hylecoetus* were more active than females while on the host. These observations suggest that females seemed to be searching for suitable oviposition sites, whereas males searched for females. After each male had located a female, the former attacked the latter by climbing on its back and engaged in copulation with both male and female facing the same direction. In some instances *Hylecoetus* landed on hosts and crawled around for a while before resuming flight. Having landed on a suitable host the majority of *Hylecoetus*, particularly females, did not resume flying.

6.1.4. Response of *Hylecoetus* to larch logs

Table 34 shows the results of the response of natural populations of *Hylecoetus* to window barrier traps baited with *Larix decidua* logs on the Atholl Estate during the 1984 season. A total of 8 adult *Hylecoetus* (sex ratio ♀1:1♂) were caught by the traps.

Table 34: RESPONSE OF *H. dermestoides* TO WINDOW BARRIER TRAPS BAITED WITH LARCH LOGS ON THE ATHOLL ESTATE IN 1984

Trap no.	Felling date of logs	Number of adults caught Female	Male	Total
1	Nov., 1983	1	0	1
2	March, 1984	1	1	2
3	March, 1984	2	3	5
4	April, 1984	0	0	0
	Total	4	4	8

6.1.5. Response of *Hylecoetus* to larch stumps

The results of the response of natural populations of *H. dermestoides* to cross vane traps baited with larch stumps are shown in Table 35. The traps were placed on top of the stumps and the *Hylecoetus* responding to the traps were removed periodically from the traps. *Hylecoetus* was also hand-picked from the surfaces of the stumps. Numbers of *H. dermestoides* collected from stumps increased from the end of April to a peak on 13 May, corresponding to the first seasonal peak. After 14 May there was no response to the stumps although *Hylecoetus* were still flying. As shown in Table 35, the numbers attracted to different stumps varied considerably, but too few observations were made for meaningful analysis.

Table 35 : RESPONSE OF *H. dermestoides* TO CROSS VANE TRAPS PLACED ON LARCH STUMPS ON THE ATHOLL ESTATE IN 1984

Flight	STUMPS								
	A	B	C	D	E	F	Total	Female	Male
29 April	1	0	0	0	0	0	1	1	0
30 April	1	0	0	0	0	0	1	1	0
2 May	2	0	0	1	0	0	3	3	0
3 May	2	0	0	0	0	0	2	2	0
13 May	5	4	0	1	2	0	12	12	0
14 May	0	0	1	0	0	0	1	0	1
Total	11	4	1	2	2	0	20	19	1

6.1.6. Response of *Hylecoetus* to *Larix decidua* standing dead trees

The response of *H. dermestoides* to 12 dying or dead larches on the Atholl Estate in 1984 is shown in Fig:40. Seven of 11 trees attracted beetles between 13—23 May, but from 24 May till 20 June only 3 of these trees remained attractive, and a twelfth tree (not included in the earlier observations) was also attractive. Females (total 215) were much more attracted than males (total 38), a difference significant at $p = 0.001$ (x^2 analysis).

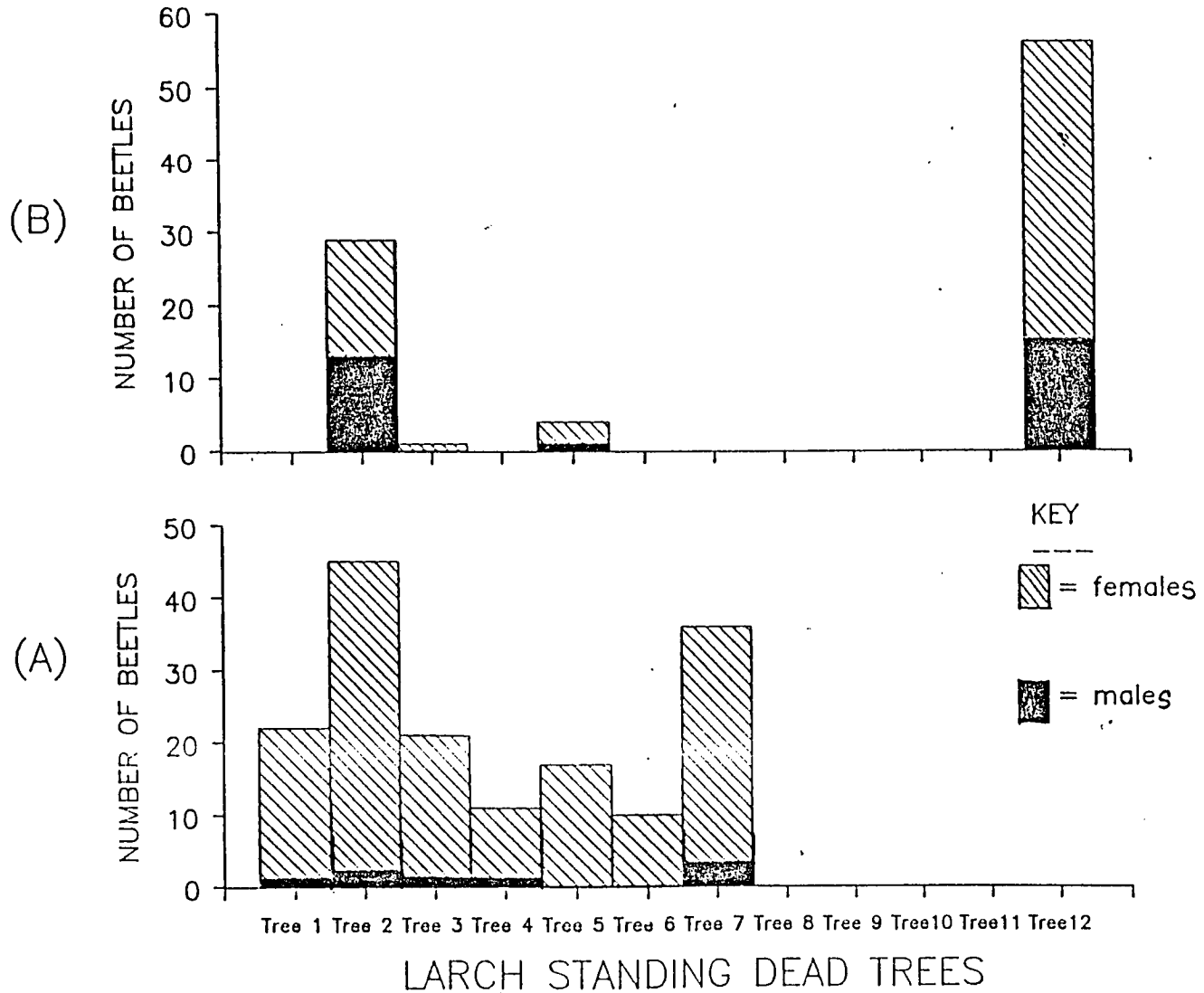


Fig:[40] The response of *Hylecoetus dermestoides* to *Larix decidua* standing dead trees on the Atholl Estates between (A) 13–23 May and (B) 24 May–30 June, 1984. 1984.

6.2. Baiting technique using ethanol as attractant

A total of 44 *Hylecoetus* (18 female and 26 male) were caught in a trap baited with ethanol which was operated from 29 May to 30 June, 1984.

6.3. Ethanol-dosage and trap type

An ethanol-dosage experiment was set up towards the end of the 1984 season. A total of 10 *Hylecoetus* (all female) were caught by the multiple funnel traps: 7 were caught by a trap with 2 vials of ethanol, and 3 were collected from with 1 vial of ethanol. *Hylecoetus* did not respond to empty (unbaited) multiple funnel traps or window barrier or cross vane traps.

6.4. Response of *Hylecoetus* to traps baited with different semiochemicals

A study was made of the response of *H. dermestoides* to window barrier traps baited with different semiochemicals set up in a Randomized Block Design between 14 May and 4 July, 1985. 21 *Hylecoetus* were collected in the 44 traps. Twelve were collected from traps baited with camphene dissolved in 95 percent ethanol; 6 beetles (all female) were collected from traps baited with ethanol; 2 beetles responded to a trap baited with camphene dissolved in chloroform, and 1 female was collected from a trap baited with chloroform. No attraction was found to β-pinene, limonene, myrcene, methanol, lineatin or to unbaited traps. The results provide preliminary evidence that ethanol attracts female *Hylecoetus*.

CHAPTER 7

CHAPTER 7

AMBROSIA BEETLE–FUNGUS SYMBIOSIS

7.1. Sources of the ambrosia fungi

Pieces of wood containing actively tended *Hylecoetus* tunnels were cut mainly from larch and spruce stumps at different sites (Map 3), between 28 September, 1985 and June, 1986. Fungal inocula obtained from the brood material was plated onto agar.

7.1.1. Larval and pupal tunnels

The walls of freshly opened *Hylecoetus* larval tunnels were found to be covered with a moist light brown layer of hyphae and bore–meal. Observation of the tunnel walls under a low power microscope (X10) revealed the presence of coremia. *Hylecoetus* larvae were observed moving through the mycelial/coremial mat and seemed to be cropping the coremia. The heads of the coremia had disappeared (Fig:41).

7.1.2. Wood slivers

Thirty five potato dextrose agar (PDA) plates were inoculated with surface sterilised wood slivers (1x1x2cm) on 28 September 1985. After 18 days incubation fungal growth had spread from the wood onto the agar on all plates. 24 (68%) of the plates had black, grey or greenish appressed mycelium. Coremia were present. Other plates were contaminated with slime and mould. The coremia and dark stained hyphae indicated the presence of *Ceratocystis* spp. Subcultures of the coremia and the dark stained hyphae gave rise to only *Ceratocystis*. Twenty corn meal agar (CMA) (2.10.1985); 18 malt extract agar and yeast extract (YEME) (1.10.1985) and 10 malt extract agar (MEA) (1.10.1985) plates were similarly inoculated with wood slivers. After two weeks incubation, only three CMA plates did not show any signs of fungal growth: the rest had

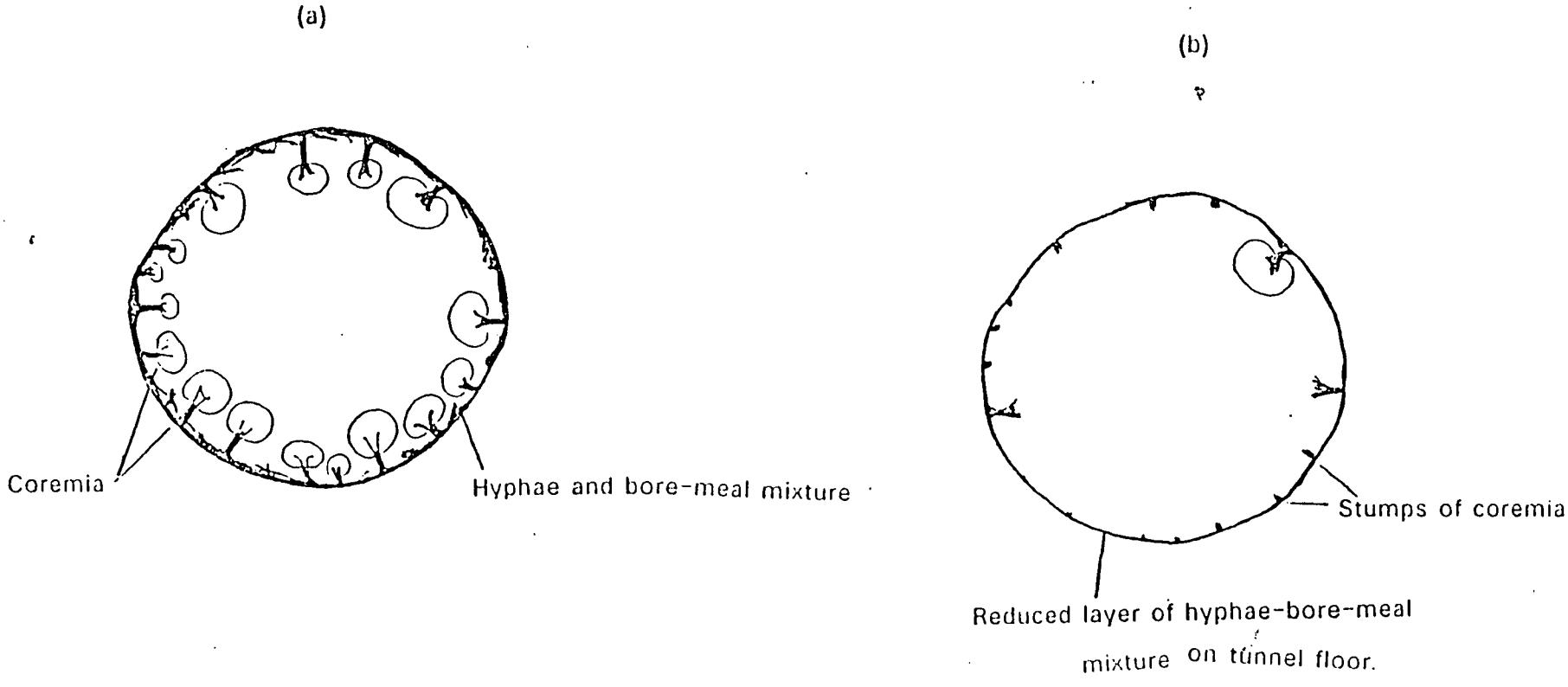


Fig:41 Cross section of a larval tunnel of *Hylecoetus* showing: (a) walls lined with a mass of fungal hyphae and bore-meal with *Ceratocystis piceae* indicated by coremia with slime globules; (b) tunnel after harvesting of fungal hyphae-coremia complex by *Hylecoetus* larva.

only *Ceratocystis*. Three MEA plates had only *Ceratocystis*, the rest had a creamy slimy bacterial growth covered with white cottony aerial mycelia. Growth on YEME was similar to that on MEA, and only two of the YEME plates had only *Ceratocystis*. Isolations of fungi from wood slivers are summarised in Table 36 and is shown in Fig:42

7.1.3. Scrapings from tunnel walls

The creamy, light brown tunnel lining was removed aseptically with a scalpel and streaked on 17 CMA, 27 YEME and 11 Bacto-agar plates. Four of the CMA, and 8 of the YEME plates had only *Ceratocystis* growth, other plates had slime (bacterial) or white-cottony aerial mycelial mat. There was no fungal growth on Bacto-agar plates (Table 36)

7.1.4. Larvae

Hylecoetus larvae freshly removed from their tunnels were in most cases covered with the creamy light brown tunnel lining. Isolations of fungi were made from the tunnel lining and from gut contents by surface sterilising the larvae before plating them onto agar plates.

Apart from the mould and slime found on some plates, only *Ceratocystis* was constantly isolated from larval trails on agar. *Ceratocystis* appeared on plates with either live or dead larvae. On PDA, MEA and YEME, coremia arose from a white-cottony mycelial growth, while growth of *C. piceae* on CMA was predominantly in the form of an almost transparent mycelial mat (Fig:43). Numerous coremia were present on the mycelial mat. Table 36 summarises information on isolations from larvae.

7.1.4.1. Dissected alimentary canals

Each surface sterilised larva was placed on a flamed glass slide and dissected with pointed needles to remove the alimentary canal. Each alimentary canal was cut into smaller lengths. The contents of the pieces of the

Table 36: SUMMARY OF INFORMATION ON THE EFFECT OF DIFFERENT INOCULA ON THE ISOLATION OF *Ceratocystis piceae* ON AGAR MEDIA

		INOCULUM					
Agar medium	*C. piceae* growth	Wood slivers	Scrapings off tunnels walls	Live larvae	Dissected alimentary canals	Bore-meal	Total
CMA[1]	Present	17	4	26	6	2	55
	Absent	3	13	16	9	3	54
	Total	20	17	42	15	15	109
PDA[2]	Present	24		12	5	7	48
	Absent	11		6	6	3	26
	Total	35		18	11	10	74
MEA[3]	Present	3		0		1	4
	Absent	7		5		10	22
	Total	10		5		11	26
YEME[4]	Present	2	8	10	9	0	29
	Absent	16	19	13	5	10	63
	Total	18	27	23	14	10	92
BACTO AGAR	Present	0	0	0	0	0	
	Absent		11	10		13	34
	Total		11	10		13	34
GRAND TOTALS	Present	46	12(58)[5]	48	20(68)[6]	10	136
	Absent	37	43(80)	50	20(70)	49	199
		83	55(138)	98	40(138)	59	335

[1] = Corn meal agar; [2] = Potato dextrose agar; [3] = malt extract agar;

[4] = malt extract agar plus yeast extract; [5] = total for tunnels and

[6] = total for larvae.

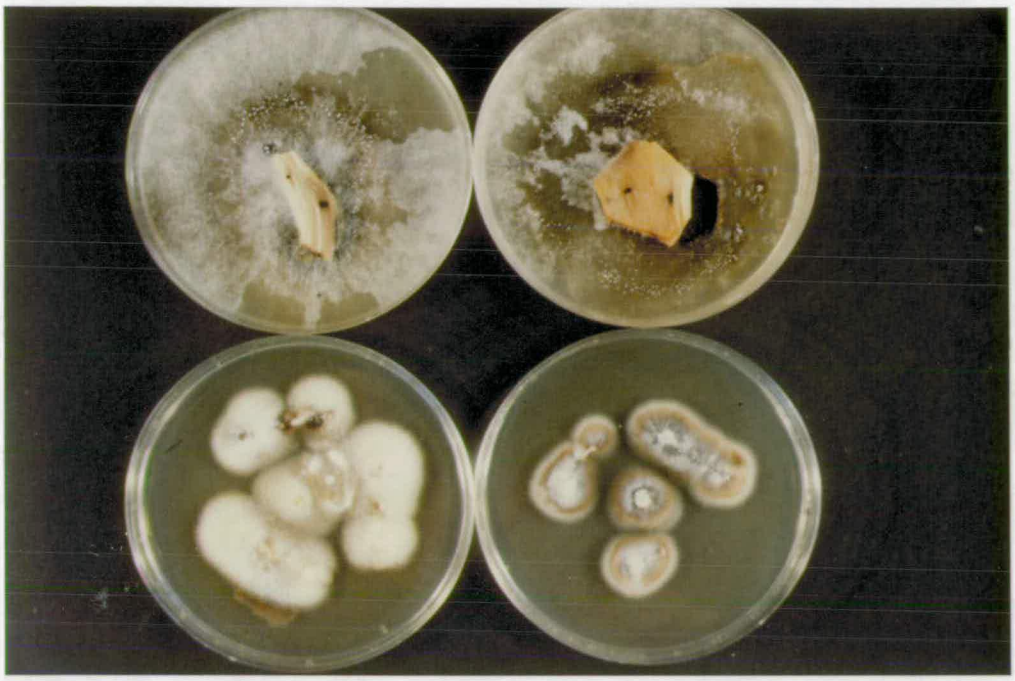


Fig: 42 Growth of ambrosia fungi from wood slivers with *Hylecoetus* larval tunnels. *Ceratocystis* growth is indicated by coremia (black stalks).

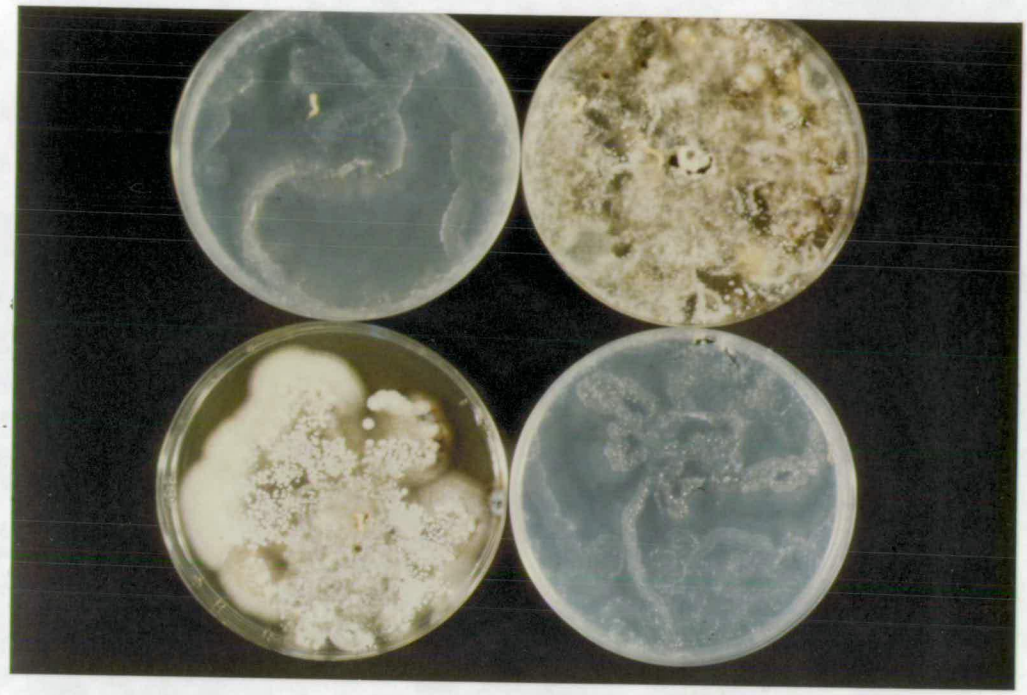


Fig:43 Growth of ambrosia fungi from traces of *Hylecoetus* larvae on agar.

digestive tract were squeezed out and streaked onto agar media, CMA, PDA and YEME. Only *Ceratocystis* was isolated (Fig:44 and Table 36).

7.1.5. Adults

Ceratocystis was isolated from female and male field collected *H. dermestoides* which were directly plated on PDA plates.

7.1.6. Eggs

All fungal isolations from eggs laid by female beetles originating from pupae on agar were unsuccessful.

7.1.7. Bore-meal

Isolations of ambrosia fungi from bore-meal of larvae of *Hylecoetus* were made on CMA, PDA, YEME and Bacto-agar. There was no fungal growth on Bacto-agar. MEA plates were covered by slime, and only one plate had *Ceratocystis*. YEME plates were all covered with mould. Both coremia and perithecia appeared on some CMA and PDA plates. Other CMA and PDA plates had slime or no fungal growth at all. Table 36 summarises information on the isolations of *Ceratocystis* from bore-meal.

7.2. Identification of ambrosia fungi

7.2.1. Preliminary findings

In addition to *Ceratocystis*, other fungi, *Phoma* spp, *Chaetomium* spp, *Gliomastix* spp, including *Cephalosporopsis* spp, Sympodulosporae and *Sphaeronaemella* spp were associated with the wood slivers. However, only *Ceratocystis* was found to be constantly associated with *Hylecoetus*.

7.2.2. Measurements of *Ceratocystis* diagnostic features

Identification of the *Ceratocystis* spp present in the different isolations was carried out using the criteria established by Bakshi (1950, 1951), Hunt (1956) and Butin (1967). Table 37 shows a comparison of attributes of the

'unknown' with known *Ceratocystis* spp. and shows that the unknown fungus corresponds to *Ceratocystis piceae*.

7.3. Factors affecting the success of isolation of ambrosia fungi

7.3.1. Effect of inoculum on isolation of ambrosia fungi

Table 36 shows that the successful isolation of *Ceratocystis* depends on the type of inoculum used. If only the total isolations are considered, the important fungal sources are wood slivers (55%), dissected alimentary canals (50%) and live larvae (49%). Scrapings of tunnel walls (22%) and bore-meal are less important. However, if isolations from wood slivers are combined with those from scrapings from tunnel walls as 'tunnels', and alimentary canal and live larvae as 'larvae', then larvae (49%) seem to be slightly better than tunnels (42%) as sources of ambrosia fungi.

7.3.2. Seasonal occurrence of ambrosia fungi in larval alimentary canals

The number of successful isolations of ambrosia fungi, expressed as a percentage of the total number of isolations made in each month was used: (1) to express the occurrence of ambrosia fungal spores in larval alimentary canals; and (2) examine the relationship between insect-fungus association and the insects' period of active tunnelling, overwintering and prepupal stages.

Both field-collected larvae and laboratory-reared larvae were studied during autumn and spring. However, only laboratory-reared larvae were studied during the winter; the larvae produced bore-meal throughout that period. Table 38 summarises information on isolations of ambrosia fungi from *Hylecoetus* larvae, carried out between September, 1985 and May, 1986. Only isolations on CMA, PDA and YEME were considered for the study.

Table 38 shows that when *Hylecoetus* larvae were actively boring into the wood, they could all have been associated with the ambrosia fungus. The

insect–fungus association decreased towards winter, and was lowest between December and January. In early spring the percentage of successful fungal isolations increased. Another decrease in the percentage of successful isolations occurred in May. This information indicates that the presence of ambrosia fungal spores in alimentary canals of *Hylecoetus* larvae seemed to be related to the different stages of larval development as follows: (1) September, larvae were actively boring into wood (feeding stage); November to January, larvae were overwintering; (3) March, some larvae resumed feeding and; (4) May, most larvae had reached the prepupal stage (spring).

Table 37: COMPARISONS OF DIAGNOSTIC FEATURES OF 'KNOWN' AND AN 'UNKNOWN' *Ceratocystis* SPECIES

[illegible]	*C. coerulescens* (Münch) Bakshi	*C. galeiformis* Bakshi	*C. piceae* (Münch) Bakshi	*C. cana* (Münch) C. Moreau	*C. flocossa*	*C. clavata* (Mathiesen) Hunt	*C. pilifera* (Mathiesen) Hunt	*C. capilifera* (Fries) C. Moreau	*C. schrenkiana* (Hedge) C. Moreau	ambrosia (Hedge) C. Moreau	'Unknown' Bakshi
[illegible] size	-	-	-	-	-	-	-	-	-	-	530.9×27.04
[illegible] base of [illegible]	250×5	2-2.5 few rarely develop in culture	120×3.5	300×2	Un-ornamented	Un-ornamented	25×3	Un-ornamented	100-3	10-50×3-4	101.4×3.7
[illegible]	2-6	1.5-5	1-3.5	1-5	5	1.5-3	2-3	1.5-3(5)	3-7	2-6	6.1
Submerged [illegible]	2-6	15.5-5	4-5	1-5	8	5.5	2-8	3-8	3-7	2-6	4.2-8.4
[illegible]	Not seen	Evanescent	Not seen	Not seen	Evanescent	Evanescent	Not seen	Not seen	Not seen	Not seen	Not seen
[illegible] growth (mm)	45	10	25	30	30	40	30	30	-	35	24(12-40)
[illegible] diameter	200	182-271.6	80-180	50-150	110-182	127-233	100-250	75-200	120-200	15-200	140±40.7
[illegible] height	-	182-273	-	-	-	127-212	-	-	-	-	104±33.3
Neck length	700	530-700	1000	1000	940-1600	453-830	2000(-3000)	600-3000	1000	420-800	85-200(424)
Neck breadth at base	35-50	33.2-60.2	20-50	20-25	35-70	28.3-45.3	20-40	20-40	15-40	20-30	11.8-53.5
Neck breadth at tip	10-20	15.4-22.0	5-25	10-15	12-20	11.3-19.8	9-16	5-21	10-20	11-16	15
[illegible] number	10-20	Lacking	15-25	Not seen	16	9-15	10-20	Numerous	Not seen	Not seen	10-20(14)
[illegible] length	20-30	-	10-25(-40)	-	10-12.3	14-31.2	20-65	40-100	-	21-25	23.04
[illegible] length	1.5-2.5	-	2-3	-	2.8	2.3	2-2.5	2-3	-	0.8-2.3	0.345-1.4
[illegible] septate or continuous	-	-	hyaline, tapered blunt at tip	-	-	-	Not described	Septate	-	1-celled rarely 2-3 celled	1-celled, hyaline tapering, pointed tips
Ascospore shape	-	Bean, kidney, crescent shaped	- -	- -	- -	Bean, kidney, crescent shaped	Bean, kidney, crescent shaped	- -	- -	- -	Broadly-ellipsoid elongate-ellipsoid quadrangular
Conidiophore type	Simple	Cephalosporium Leptographium	Graphium	Cephalosporium Graphium	Graphium + undifferentiated	Graphium + undifferentiated	Cladosporium	Simple	Simple tree-like tuft	Simple	Cladosporium Graphium, Cephalosporium

All measurements of length or diameter are in μ.

Table 38: SEASONAL OCCURRENCE OF *Ceratocystis piceae* IN *Hylecoetus* LARVAE FROM ATHOLL ESTATE, IN 1985-86

Date of Isolation	Total number Of cultures Plated	Number of Successful Isolations	% Successful Isolations
Sept.,1985	10	10	100.00
Oct., 1985	56	35	62.50
Nov., 1985	65	30	46.15
Dec., 1985	6	1	16.67
Jan., 1986	40	11	27.50
March, 1986	105	71	67.62
May,1986	31	16	51.61.

CHAPTER 8

CHAPTER 8

WOOD AS SOURCE OF NUTRIENTS

8.1. Thin layer chromatography

Preliminary studies using standard solutions of amino acids indicated that separation efficiency, i.e. good spot separation and colour development was by far the best on cellulose 144, 250μ thick layers, with n-butanol-glacial acetic acid-water, and phenol-water in the first and second phases respectively (Stahl, 1965; Randerath, 1968).

Amino acids in bark, wood and bore-meal extracts were identified by comparing colours and R_f values of the developed spots with those of the known standards (Table 39). The R_f value was determined as described by Stahl (1965) and Randerath (1968).

$$R_f = \frac{\text{distance travelled by spot}}{\text{distance travelled by solvent front}}$$

The distance of the centre of each spot from the starting point was computed as the mean of the upper and lower front. A summary of the results of thin layer chromatography (TLC) of amino acids in larch bark and wood is presented as follows:

1. Figs 44 and 45, show one-dimensional TLC of inner bark free amino acids extract obtained by using cold 70 percent ethanol. Five spots developed from the sample, and these corresponded to 13 known amino acids (Table 40).

2. Fig:46, shows the two-dimensional TLC of free amino acids extracted from sapwood. Seven and 13 spots were developed in the first and second dimensions respectively. The 13 spots corresponded to 17 known amino acids (Table 41).

3. Fig:47 and Table 42 show the two-dimensional TLC of free

amino acids extracted from sapwood by using boiling 80 percent ethanol. Eleven spots were developed in the second direction, and these corresponded to 18 known amino acids.

4. Fig:48, gives information on the two-dimensional TLC of protein-bound amino acids extracted from larch sapwood. Nine spots were developed after running the plate in the second dimension, and 8 of the spots corresponded to 12 known amino acids. The remaining spot was unidentified (Table 43).

5. Table 44 summarises the results of TLC of free and protein-bound amino acids extracted from larch bark and sapwood, and shows that most common amino acids were present in both bark and wood extracts. However, valine, leucine, gamma-amino butyric acid and 4-gamma-amino butyric acid, were not detected in the bark extract. Phenylalanine was not detected in the cold 70 percent ethanol-soluble bark and wood extracts, and cystine was not detected in the cold 70 percent ethanol-soluble wood extract, and proline and tyrosine were absent in the boiling 80 percent ethanol-soluble wood extract. There were slightly more free than protein-bound amino acids in the extracts.

8.2. Quantitative composition of amino acids in bark, sapwood and bore-meal

8.2.1. Paper electrophoresis

Figs 49 and 50, and Table 45 summarise information on the separation of free amino acids in larch bark and *Hylecoetus* bore-meal, by High Voltage Electrophoresis (H.V.E.P.). There did not seem to be any difference in the numbers of amino acids detected in both bark and bore-meal, and most of the common amino acids were found in both samples (Fig:49). However, a greater number of free amino acids was found in the frass extract than the bark extract (Table 45).

8.2.2. Ion-exchange chromatography

The results of the quantitative composition of amino acids in water-soluble nitrogen fractions in larch bark (Fig:51), sapwood (Fig:52) and bore-meal (Fig:53-54) are summarised in Tables 46, 47, 48 and 49 respectively. Only amino acids extracted from bark and sapwood of one tree were analysed.

Aspartic acid and glycine only were found in the wood extract (Table 49). No basic amino acids, such as lysine, histidine and arginine were found in the bark extract (Fig:51, Table 46). However, all these basic amino acids and 14 neutral and acidic acids, were found in the bore-meal extract (Fig:54, Table 48). In the bark extract, the major components were glycine, alanine, valine and serine. The major components of the bore-meal extract were alanine, glycine, serine and threonine. Glycine and alanine were the major components in the bark and bore-meal extracts respectively.

8.3. TLC of sugars from sapwood and bore-meal extracts

Tables 50 and 51 summarize the results of TLC of sugars in wood and *H. dermestoides* bore-meal extracts. There does not seem to be a significant difference between the qualitative amounts of sugars found in fresh wood and *Hylecoetus* frass. Glucose, fructose, galactose, sucrose, raffinose and maltose were the main sugars found in the extracts examined.

Table 39 : TWO DIMENSIONAL TLC OF STANDARD AMINO ACIDS ON CELLULOSE 144 LAYERSWITH N-BUTANOL-GLACIAL ACETIC ACID-WATER AND PHENOL-WATER AS SOLVENTS

NO.	AMINO ACID	UPPER FRONT (cm)	LOWER FRONT (cm)	R_f
1	Glycine	2.0	1.3	0.165
2	Serine	2.0	0.9	0.145
3	Alanine	3.0	2.2	0.260
4	Valine	5.2	3.8	0.450
5	Leucine	6.6	5.1	0.585
6	Threonine	2.1	1.5	0.180
7	Aspartic acid	2.3	1.1	0.170
8	Arginine	1.3	0.0	0.065
9	Histidine	2.3	1.3	0.180
10	Hydroxy-proline	2.3	1.3	0.180
11	Lysine	1.1	0.0	0.055
12	Phenylalanine	5.7	4.8	0.525
13	Proline	3.0	2.4	0.270
14	Tryptophane	4.2	3.3	0.375
15	Tyrosine	3.5	2.8	0.315
16	Cystine	1.0	0.0	0.050
17	α-amino butyric acid	2.7	1.9	0.230
18	Asparagine	1.3	0.4	0.085
19	4-amino butyric acid	2.8	2.0	0.240

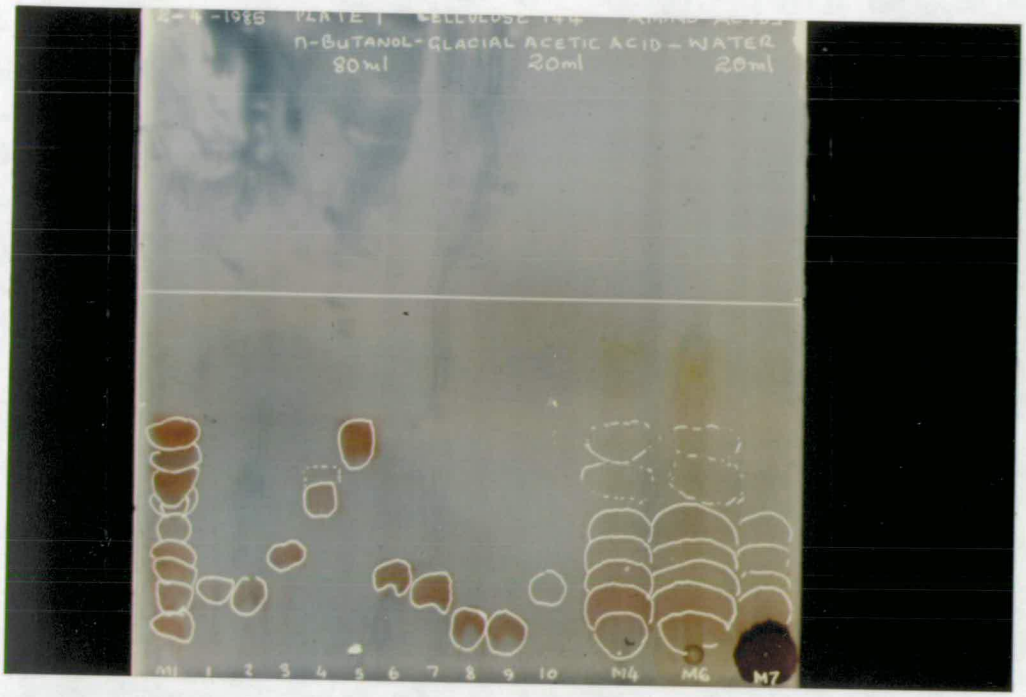


Fig:44 One-dimensional thin-layer chromatography (TLC) of larch inner bark free amino acids extract obtained by using cold 70% ethanol.

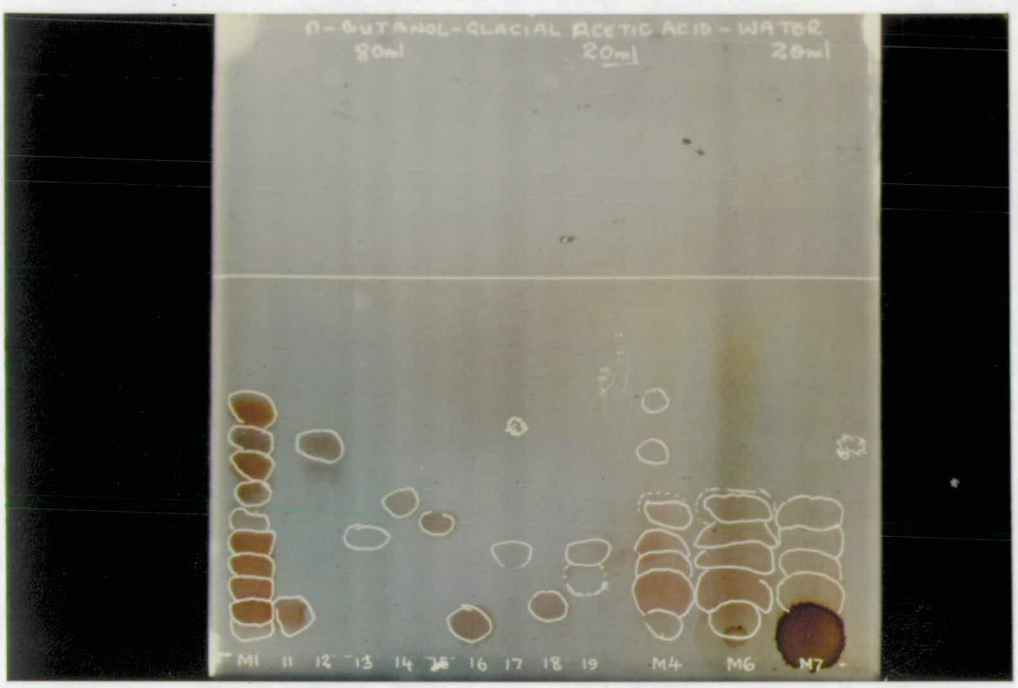
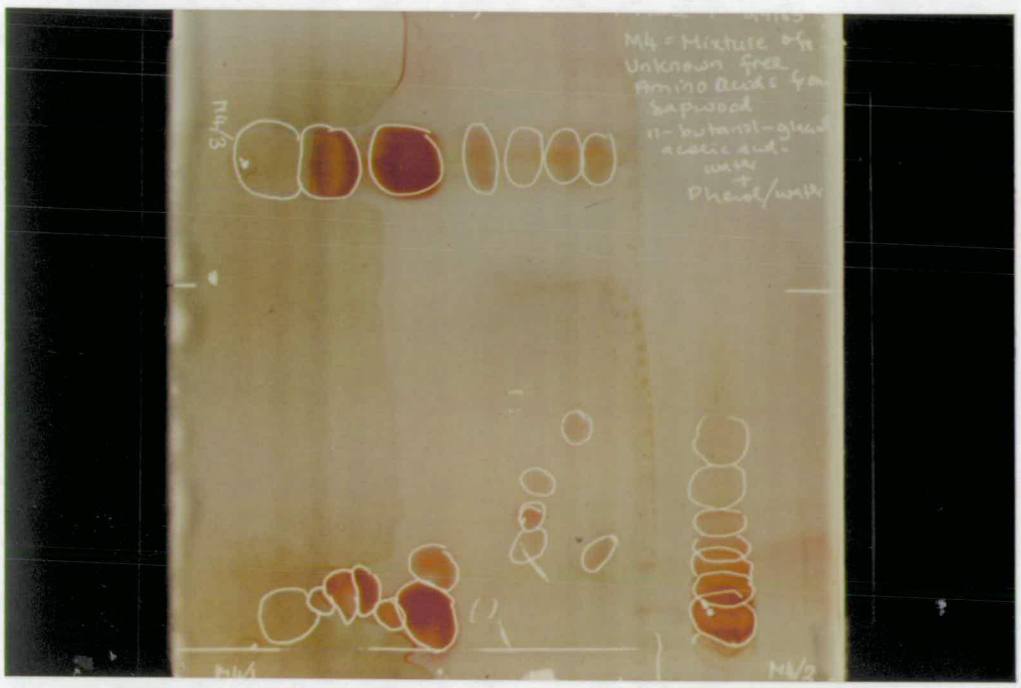


Fig:45 One-dimensional thin-layer chromatography (TLC) of larch inner bark free amino acids extract obtained by using cold 70% ethanol.

Table 40 : ONE-DIMENSIONAL THIN LAYER CHROMATOGARPHY (TLC) OF FREE-AMINO ACIDS IN LARCH BARK EXTRACT ON CELLULOSE 144 LAYERS WITH N-BUTANOL-GLACIAL ACETIC ACID-WATER AS SOLVENT (solvent front = 10cm)

AMINO ACID SPOTS ON PLATE 4					STANDARD AMINO ACIDS ON PLATE 4				CORRESPONDING AMINO ACID
Spot no.	Plate no.	Upper front (cm)	Lower front (cm)	R_f	Spot no.	Upper front (cm)	Lower front	R_f	
1	1	1.2	0.0	0.060	8	1.3	0.0	0.065	Arginine
					9	1.2	0.0	0.060	Histidine
	2	0.6	0.0	0.030	11	1.1	0.0	0.055	Lysine
					16	1.0	0.0	0.050	Cystine
					18	1.3	0.4	0.085	Asparagine
2	1	1.9	0.9	0.140	1	2.0	1.3	0.165	Glycine
	2	1.8	0.60	0.120	2	2.0	1.5	0.145	Serine
3	1	2.3	1.7	0.200	6	2.1	1.5	0.180	Threonine
	2	2.4	1.5	0.195	7	2.3	1.1	0.170	Aspartic acid
					10	2.3	1.3	0.180	Hydroxy-proline
4	1	3.1	2.3	0.270	3	3.0	2.2	0.260	Alanine
	2	3.0	2.7	0.285	13	3.0	2.4	0.270	Proline
5	1	4.1	3.0	0.355	14	4.2	3.3	0.375	Tryptophane
	2	3.9	3.0	0.345					

Second dimension



First dimension

Fig:46 Two-dimensional TLC of free-amino acids extracted from larch sapwood.

M4/1, M4/2 and M4/3 are samples of a mixture of amino acids in a sapwood extract. M4/1 was run in the first and second dimensions. Distances travelled by spots developed from M4/1 after the second dimension were measured starting with the lowest (X=spot 1, Table 41) and ending with highest (Y=spot 13, Table 41). M4/2 was run only in the first dimension and M4/3 was run only in the second dimension.

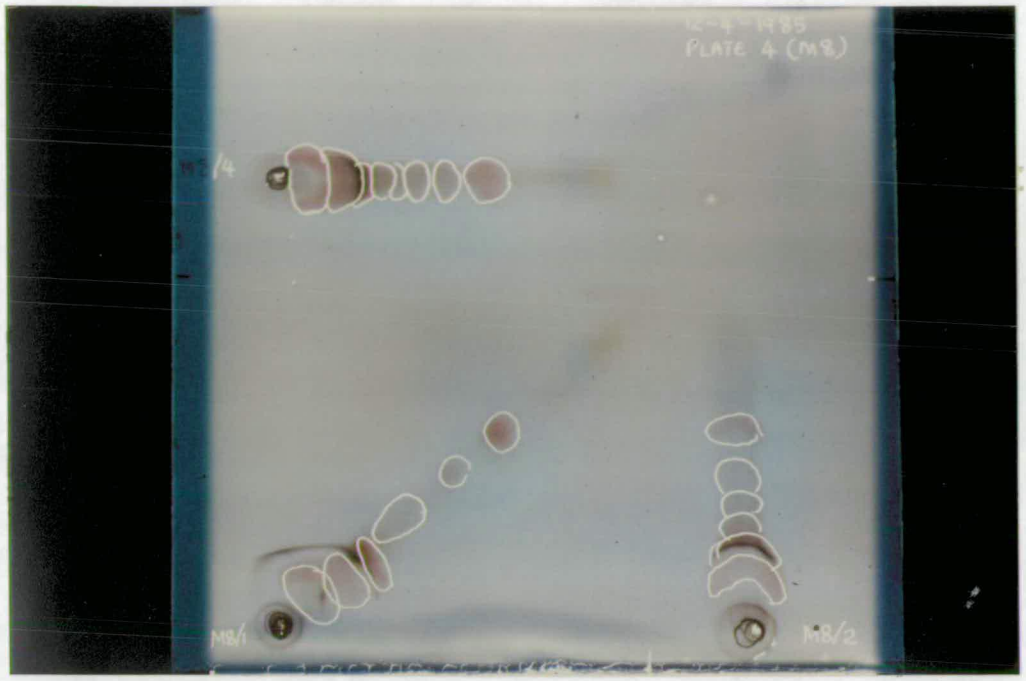
Table 41 : TWO-DIMENSIONAL THIN-LAYER CHROMATOGRAHY (TLC) FREE-AMINO ACIDS IN LARCH SAPWOOD EXTRACT ON CELLULOSE 144 LAYERS WITH N-BUTANOL-GLACIAL ACETIC ACID-WATER, AND PHENOL-WATER AS SOLVENTS (solvent front = 10cm)

AMINO ACID SPOTS ON PLATE 4				STANDARD AMINO ACIDS ON PLATE 4				CORRESPONDING AMINO ACID
Spot no.	Upper front (cm)	Lower front (cm)	R_f	Spot no.	Upper front (cm)	Lower front	R_f	
1	1.2	0.5	0.095	18	1.3	0.4	0.085	Asparagine
2	1.5	0.7	0.110	8	1.3	0.0	0.065	Arginine
3	1.7	0.2	0.095	11	1.1	0.0	0.055	Lysine
4	1.7	0.9	0.130	9	1.20	0.0	0.060	Histidine
5	2.0	0.0	0.100	2	2.0	0.9	0.145	Serine
6	2.1	0.7	0.140	1	2.0	1.3	0.165	Glycine
7	2.3	0.9	0.160	6	2.1	1.5	0.180	Threonine
				7	2.3	1.1	0.170	Aspartic acid
				10	2.3	1.3	0.180	Hydroxy-proline
8	2.9	1.7	0.230	17	2.7	1.9	0.230	γ-amino butyric acid
				19	2.8	2.0	0.240	4-amino butyric acid
9	3.2	2.2	0.270	3	3.0	2.2	0.260	Alanine
				13	3.0	2.4	0.270	Proline
10	3.3	2.5	0.290	15	3.5	2.8	0.135	Tyrosine
11	4.0	3.2	0.360	14	4.2	3.3	0.375	Tryptophane
12	5.0	4.2	0.460	4	5.2	3.8	0.450	Valine
13	6.6	5.7	0.615	5	6.6	5.1	0.585	Leucine

Fig:47 Two-dimensional TLC of free-amino acids extracted from larch sapwood

M6/1 M6/2 and M6/3 are samples of a mixture of amino acids in a sapwood extract. M6/1 was run in the first and second dimensions. Distances travelled by spots developed from M6/1 after the second dimension were measured starting with the lowest (X=spot 1, Table 42) and ending with highest (Y=spot 11 Table 42). M6/2 was run only in the first dimension and M6/3 was run only in the second dimension.

Second dimension →



First dimension →

Fig: 48 Two-dimensional TLC of protein-bound amino acids extracted from larch sapwood.

M8/1 M8/2 and M8 M8/1, M8/2 and M8/3 are samples of a mixture of amino acids in a sapwood extract. M8/1 was run in the first and second dimensions. Distances travelled by spots developed from M8/1 after the second dimension were measured starting with the lowest (X=spot 1, Table 43) and ending with highest (Y=spot 9, Table 43). M8/2 was run only in the first dimension and M8/3 was run only in the second dimension.

Table 42 : TWO-DIMENSIONAL THIN LAYER CHROMATOGARPHY (TLC) OF FREE-AMINO ACIDS IN LARCH SAPWOOD EXTRACT ON CELLULOSE 144 LAYERS WITH N-BUTANOL-GLACIAL ACETIC ACID-WATER, AND PHENOL-WATER AS SOLVENTS (solvent front = 10cm)

AMINO ACID SPOTS+[1] ON PLATE 1				STANDARD AMINO ACIDS[2] ON PLATE 1				CORRESPONDING AMINO ACID
Spot no.	Upper front (cm)	Lower front (cm)	R_f	Spot no.	Upper front (cm)	Lower front	R_f	
1	1.0	0.5	0.075	8	1.3	0.0	0.065	Arginine
				9	1.2	0.0	0.060	Histidine
				11	1.1	0.0	0.055	Lysine
				16	1.0	0.0	0.050	Cystine
				18	1.3	0.4	0.085	Asparagine
2	1.8	0.1	0.095	2	2.0	0.9	0.145	Serine
3	1.8	0.5	0.115	1	2.0	1.3	0.165	Glycine
4	2.1	0.5	0.130	6	2.1	1.5	0.180	Threonine
				7	2.3	1.1	0.170	Aspartic acid
				10	2.3	1.3	0.180	Hydroxy-proline
5	2.6	1.9	0.225	17	2.7	1.9	0.230	γ-amino butyric acid
6	2.8	1.8	0.230	19	2.8	2.0	0.240	4-amino butyric acid
7	3.2	1.3	0.225	3	3.0	2.2	0.260	Alanine
				13	3.0	2.4	0.270	Proline
8	4.2	2.5	0.335	14	4.2	3.3	0.375	Tryptophane
9	5.6	4.5	0.505	4	5.2	3.8	0.450	Valine
10	5.6	4.7	0.515	12	5.7	4.8	0.525	Phenylalanine
11	6.1	5.0	0.555	5	6.6	5.1	0.585	

Table 43 : TWO-DIMENSIONAL THIN LAYER CHROMATOGARPHY (TLC) OF BOUND-AMINO ACIDS IN LARCH SAPWOOD EXTRACT ON CELLULOSE 144 LAYERS WITH N-BUTANOL-GLACIAL ACETIC ACID-WATER, AND PHENOL-WATER AS SOLVENTS (solvent front = 10cm)

AMINO ACID SPOTS ON PLATE 4				STANDARD AMINO ACIDS ON PLATE 4				CORRESPONDING AMINO ACID
Spot no.	Upper front (cm)	Lower front (cm)	R_f	Spot no.	Upper front (cm)	Lower front	R_f	
1	1.8	0.9	0.135					
2	2.1	0.6	0.1350	1	2.0	1.3	0.165	Glycine
3	2.1	0.6	0.135	1	2.0	1.3	0.165	Serine
4	2.2	1.5	0.185	6	2.1	1.5	0.180	Threonine
				10	2.3	1.3	0.180	Hydroxy-proline
5	2.3	0.5	0.140	7	2.3	1.11	0.170	Aspartic
6	2.7	2.0	0.235	3	3.0	2.2	0.260	Alanine
				13	3.0	2.4	0.270	Proline
				17	2.7	1.9	0.230	γ-amino butyric acid
				19	2.8	2.0	0.240	4-amino butyric acid
7	5.0	3.6	0.430	4	5.2	3.8	0.450	Valine
8	5.3	4.6	0.495	12	5.7	4.8	0.525	Phenylalanine
9	6.7	5.4	0.605	5	6.6	5.1	0.585	Leucine

Table 44 : SUMMARY OF RESULTS OF TLC OF FREE AND PROTEIN BOUND AMINO ACIDS IN LARCH BARK AND SAPWOOD EXTRACTS

No.	AMINO ACIDS AND THE EXTRACTS IN WHICH THEY WERE FOUND Cold 70% ethanol-bark extract (Table21)	Cold 70% ethanol-wood extract (table22)	Boiling 80% ethanol-wood extract (Table23)	Protein bound amino acid extract from wood(Table24)
1	Glycine	Glycine	Glycine	Glycine
2	Serine	Serine	Serine	Serine
3	Alanine	Alanine	Alanine	Alanine
4		Valine	Valine	Valine
5		Leucine	Leucine	Leucine
6	Threonine	Threonine	Threonine	Threonine
7	Aspartic acid	Aspartic acid	Aspartic acid	Aspartic acid
8	Arginine	Arginine	Arginine	
9	Histidine	Histidine	Histidine	
10	Hydroxy-proline	Hydroxy-proline	Hydroxy-proline	Hydroxy-proline
11	Lysine	Lysine	Lysine	
12			Phenylalanine	Phenylalanine
13	Proline	Proline	Proline	Proline
14	Tryptophane	Tryptophane	Tryptophane	
15	Tyrosine	Tyrosine		
16	Cystine		Cystine	
17		γ-amino butyric acid	γ-amino butyric caid	γ-amino butyric caid
18	Asparagine	Asparagine	Asparagine	
19		4-amino butyric acid	4-amino butyric acid	4-amino butyric acid

Direction of development

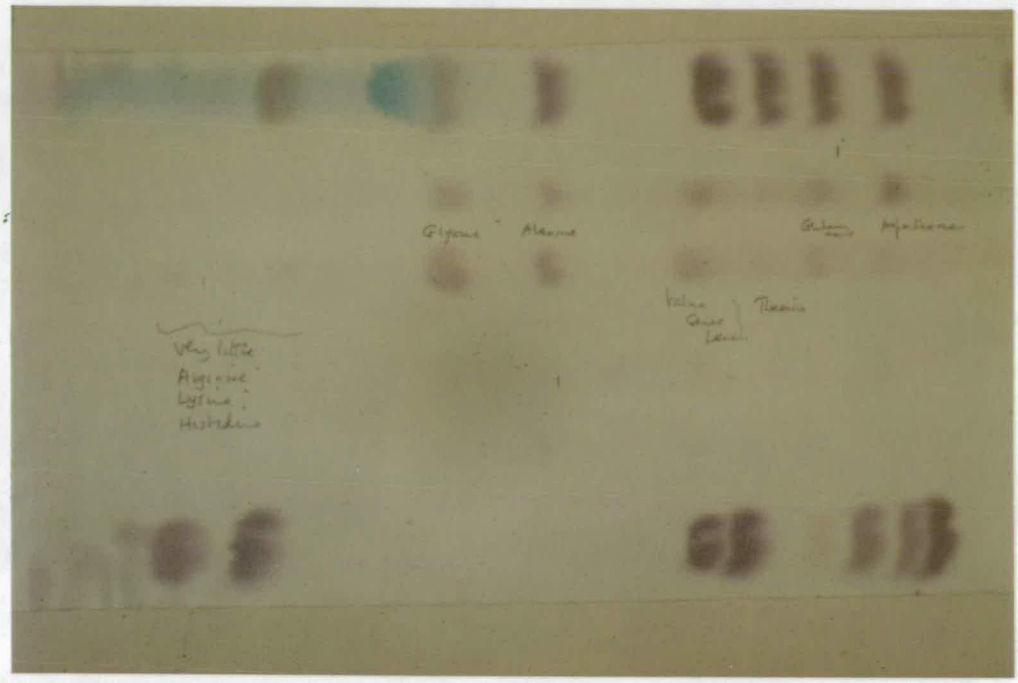


Fig:49 Separation of amino acids in larch bark extracts by High Voltage Electrophoresis (H.V.E.P.).

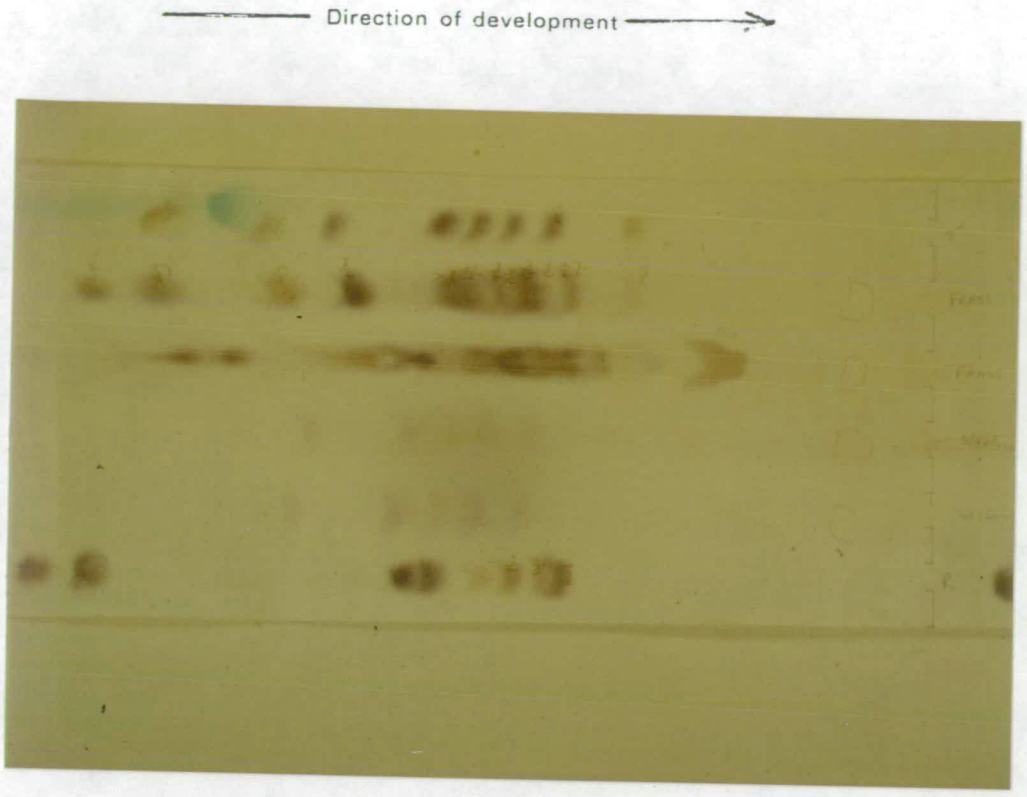


Fig:50 Separation of amino acids in *Hylecoetus* bore-meal by High Voltage Electrophoresis.

Table 45 : PAPER ELECTOPHORESIS OF AMINO ACIDS IN LARCH BARK AND *Hylecoetus* BORE-MEAL

AMINO ACIDS AND THE EXTRACTS IN WHICH THEY WERE FOUND			
No.	Frass extract (1)	Frass extract (2)	Bark extract
1		Tyrosine	
2	Aspartic acid	Aspartic acid	Aspartic acid
3		Phenylalanine	
4	Glutamic acid	Glutamic acid	Glutamic acid
5		Proline	
6	Threonine	Threonine	Threonine
7	Leucine	Leucine	Leucine
8		*iso*-Leucine	
9	Serine	Serine	Serine
10	Valine	Valine	Valine
11	Alanine	Alanine	Alanine
12	Glycine	Glycine	Glycine
13	Arginine	Arginine	Arginine
14	Lysine	Lysine	Lysine
15	Histidine		Histidine

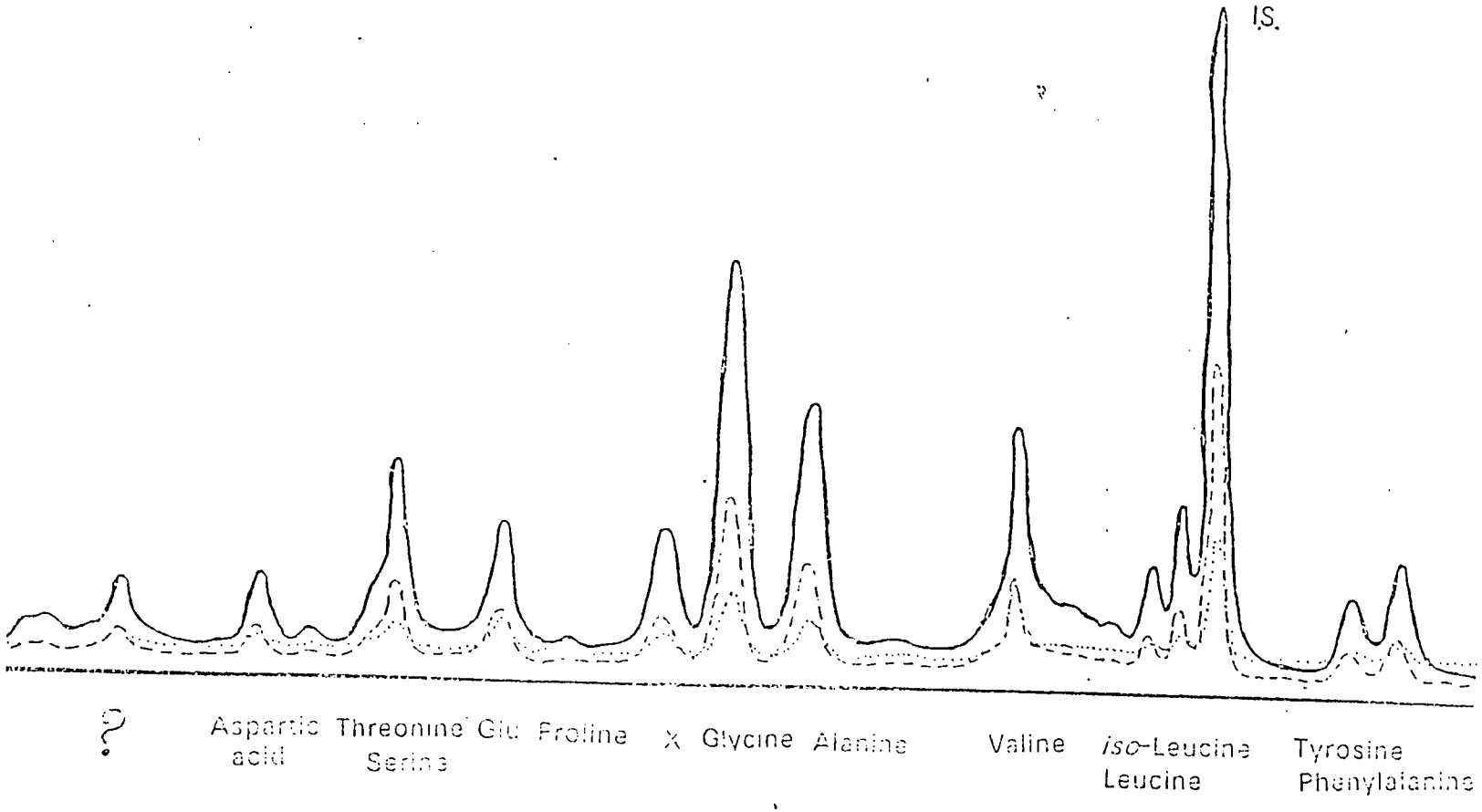


Fig:51 Quantitative composition of amino acids in water-soluble nitrogen fractions in larch bark **(traced from Amino Acid Analyser Charts then reduced)**

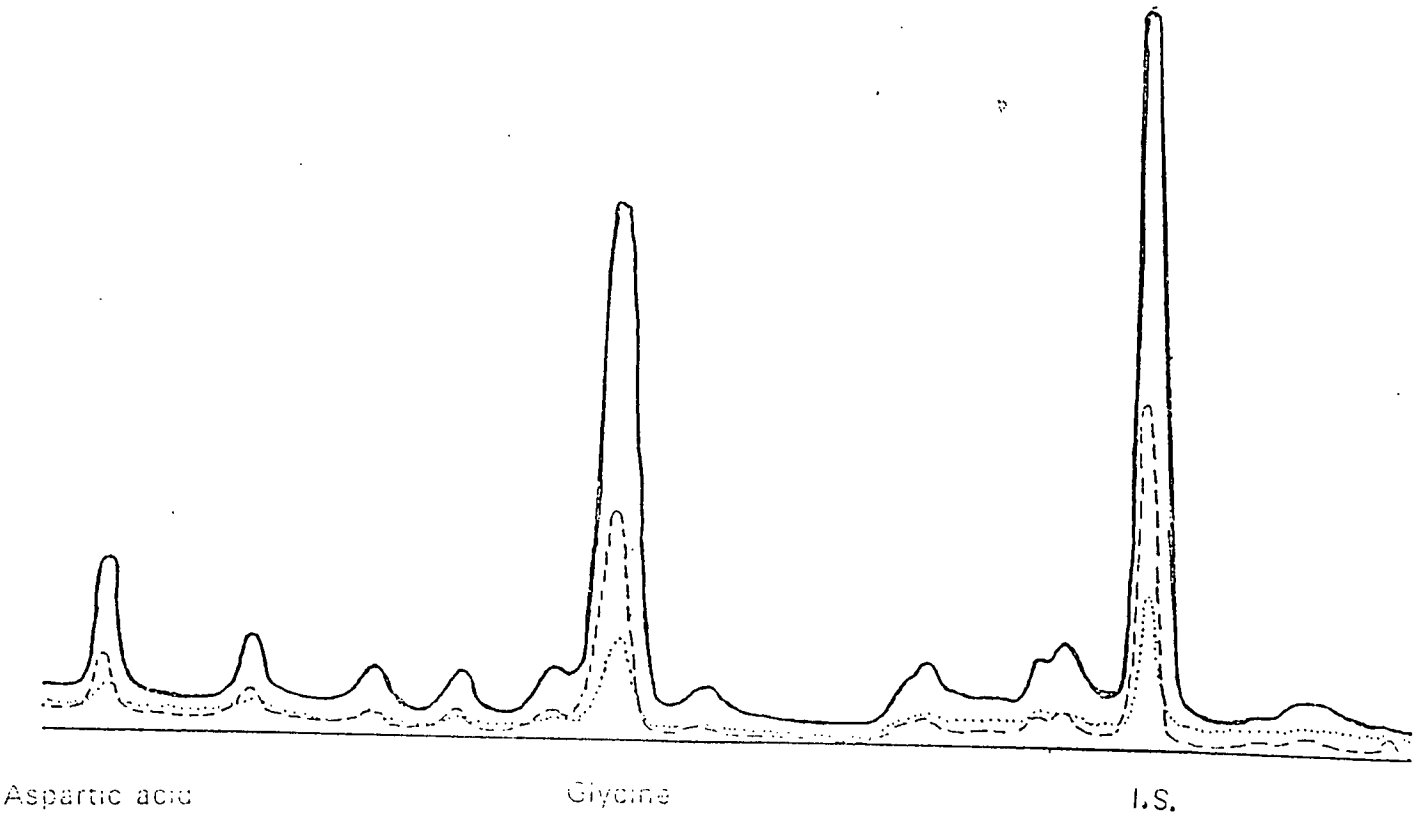


Fig:52 Quantitative composition of amino acids in water-soluble nitrogen fractions in larch sapwood (traced from Amino Acid Analyser Charts then reduced)

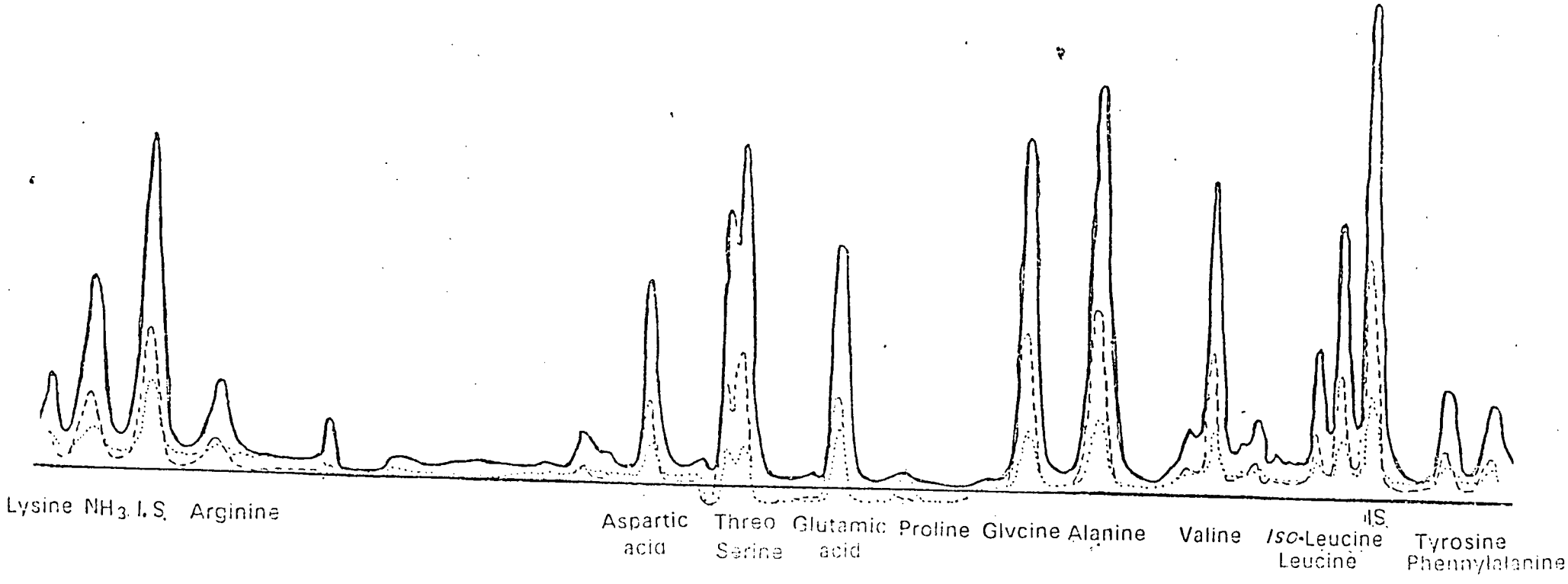


Fig:53 Quantitative composition of amino acids in water-soluble nitrogen fractions in *Hylecoetus* bore-meal.(traced from Amino Acid Analyser Charts then reduced)

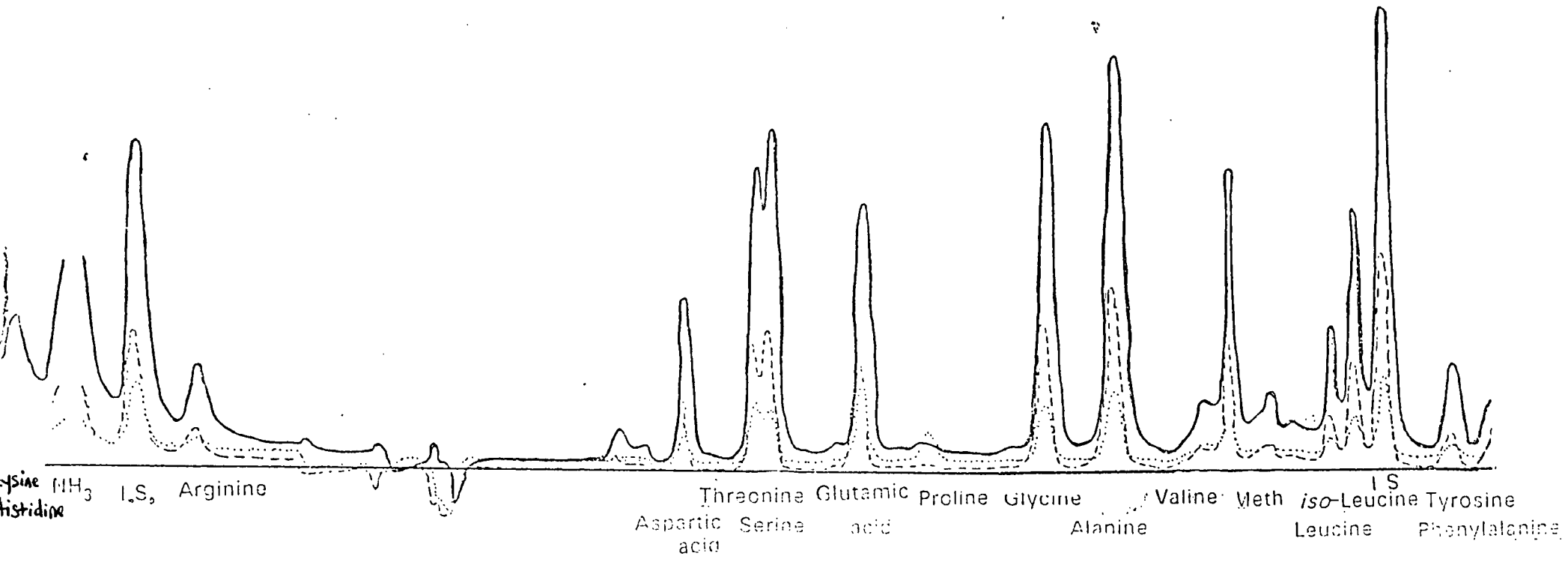


Fig:54 Quantitative composition of amino acids in water-soluble nitrogen fractions in *Hylecoetus* bore-meal (traced from Amino Acid Analyser Charts then reduced)

Table 46: QUANTITATIVE AMINO ACID COMPOSITION OF LARCH BARK EXTRACT (SAMPLE T-4)

No.	Amino acid	Base Line	Height	Half Height	H Net Height	W Width (Dots)	Micromoles (HxW)/C
1	Lysine						
2	Histidine						
3	Arginine						
4	Aspartic acid	0.013	0.037	0.025	0.0.24	12.4	0.0065
5	Threonine					x[1]	
6	Serine	0.017	0.081	0.049	0.064	13.5	0.0183
7	Glutamic acid	0.018	0.058	0.038	0.040	13.8	0.0120
8	Proline				0.005	~20	0.0085
9	Glycine	0.020	0.173	0.097	0.153	14.9	0.0500
10	Alanine	0.017	0.107	0.062	0.090	16.6	0.0318
11	Valine	0.016	0.098	0.057	0.082	11.4	0.0192
12	Isoleucine	0.022	0.046	0.034	0.024	11.2	0.0056
13	Leucine	0.018	0.071	0.045	0.053	11.5	0.0124
14	Tyrosine	0.012	0.036	0.024	0.024	15.3	0.0079
15	Phenylalanine	0.012	0.049	0.031	0.037	15.0	0.0116

Table 47: QUANTITATIVE AMINO ACID COMPOSITION OF *Hylecoetus* FRASS (SAMPLE 1)

No.	Amino acid	Base Line	Height	Half Height	H Net Height	W Width (Dots)	Micromoles (HxW)/C
1	Lysine					x[1]	
2	Histidine	0.006	0.047	0.270	0.041	14.0	0.0127
3	Arginine	0.010	0.046	0.028	0.036	17.8	0.0142
4	Aspartic acid	0.007	0.106	0.057	0.099	11.5	0.0250
5	Threonine	0.006	0.150	0.078	0.144	~11.4	0.0355
6	Serine	0.004	0.196	0.100	0.192	~10.0	0.0407
7	Glutamic acid	0.006	0.130	0.068	0.124	12.0	0.0323
8	Proline	0.008	0.008	0.000	0.016	16.0	0.0127
9	Glycine	0.006	0.206	0.106	0.200	13.3	0.0583
10	Alanine	0.008	0.249	0.129	0.241	14.3	0.0733
11	Valine	0.022	0.177	0.100	0.155	08.6	0.0274
12	Isoleucine	0.017	0.077	0.047	0.060	09.9	0.0125
13	leucine	0.017	0.154	0.086	0.137	11.2	0.0313
14	Tyrosine	0.008	0.055	0.032	0.047	14.4	0.142
15	Phenylalanine	0.006	0.048	0.027	0.042	15.6	0.0137

Table 48 : QUANTITATIVE AMINO ACID COMPOSITION OF *Hylecoetus* FRASS (SAMPLE 2)

No.	Amino acid	Base Line	Height	Half Height	H Net Height	W Width (Dots)	Micromoles (HxW)/C
1	Lysine	0.030	0.156	0.093	0.126	10.6	0.0263
2	Histidine	0.030	0.060	0.045	0.030	~11.3	0.0075
3	Arginine	0.020	0.053	0.037	0.033	15.3	0.0122
4	Aspartic acid	0.005	0.090	0.048	0.085	09.7	0.0181
5	Threonine	0.006	0.172	0.089	0.166	12.0	0.0431
6	Serine	0.008	0.198	0.103	0.190	12.2	0.0491
7	Glutamic acid	0.010	0.148	0.079	0.138	11.9	0.0357
8	Proline	0.004	0.018	0.011	0.014	~16.0	0.0190
9	Glycine	0.010	0.206	0.108	0.196	13.0	0.0559
10	Alanine	0.013	0.261	0.137	0.248	14.0	0.0739
11	Valine	0.024	0.173	0.099	0.149	08.8	0.0269
12	Methionine	0.023	0.041	0.032	0.018	10.8	0.0042
13	Iso-leucine	0.020	0.076	0.048	0.056	10.2	0.0120
14	Leucine	0.020	0.148	0.084	0.128	11.6	0.0302
15	Tyrosine	0.013	0.055	0.034	0.042	13.7	0.121
16	Phenylalanine	0.008	0.048	0.028	0.040	15.6	0.0130

Table 49 : QUANTITATIVE AMINO ACID COMPOSITION OF LARCH SAPWOOD EXTRACT (SAMPLE T-4)

No.	Amino acid	Base Line	Height	Half Height	H Net Height	W Width (Dots)	Micromoles (HxW)/C
1	Lysine						
2	Histidine						
3	Arginine						
4	Aspartic acid	0.013	0.057	0.035	0.0.44	11.8	0.114
5	Threonine						
6	Serine						
7	Glutamic acid						
8	Proline						
9	Glycine	0.012	0.210	0.111	0.198	17.6	0.0764
10	Alanine						
11	Valine						
12	Isoleucine						
13	Leucine						
14	Tyrosine						
15	Phenylalanine						

Table 50: ONE-DIMENSIONAL TCL OF SUGARS FROM LARCH SAPWOOD AND *Hylecoetus* BORE-MEAL WITH N-BUTANOL-GLACIAL ACETIC ACID-DIETHYLETHER-WATER (9:6:3:1) AS SOLVENT

SUGAR SPOTS DEVELOPED FROM EXTRACTS		STANDARD SUGARS							
Extract	spot no.	R_{f1}	R_{f2}	R_{f3}	R_{f4}	R_{f5}	R_{f6}	R_{f7}	Corresponding sugar
Larch sapwood	1	0.59					0.60	0.50	Glucose
							0.57	0.58	Fructose
							0.51	0.52	Galactose
							0.56		Maltose
	2	0.45	0.47				0.42	0.45	Sucrose
	3	3.0	0.28	0.28			0.20	0.26	Raffinose
Bore-meal	1	0.55	0.55	0.57	0.60	0.5	0.60	0.50	Glucose
							0.57	0.58	Fructose
							0.52	0.52	Galactose
							0.56		Maltose
	2	0.37	0.37	0.38	0.37	0.35	0.42	0.45	Sucrose
	3	0.14	0.10	0.09	0.06	0.100	0.20	0.26	Raffinose

Table 51: TWO-DIMENSIONAL TCL OF SUGARS FROM LARCH SAPWOOD WITH N-BUTANOL-GLACIAL ACETIC ACID-DIETHYLETHER-WATER (9:6:3:1) AND N-BUTANOL-ACETONE-WATER (4:5:1) AS SOLVENTS

SUGAR SPOTS DEVELOPED FROM PLANT EXTRACT		STANDARD SUGARS			CORRESPONDING SUGAR
	Spot no.	R_{f1}	R_{f2}	R_f	
Larch sapwood	1	0.45	0.47	0.45	Glucose
				0.48	Fructose
				0.44	Galactose
				0.43	Sucrose
	2	0.28	0.28	0.30	Maltose
				0.30	Raffinose
	3	0.05	0.05		Unknown

N.B. no sugar spots were developed from bore-meal extracts in the second dimension

CHAPTER 9

CHAPTER 9

GROWTH STUDIES ON AMBROSIA FUNGUS

This section deals with the results of an investigation designed to establish certain nutritional and physical parameters for optimum growth of *Ceratocystis piceae* an ambrosia fungus for *Hylecoetus dermestoides*. Interest was devoted to carbon and minerals i.e. nitrogen nutrients since these have been shown to be necessary for fungal growth. The influence of other factors of major importance for *Ceratocystis* growth, i.e. temperature and pH were studied. Solid media cultures were used.

9.1. Effect of agar media and sapwood/agar

Growth of *C. piceae* occurred on malt extract plus yeast extract agar (YEME), potato dextrose agar (PDA), corn meal agar (CMA), Czapek dox agar (CzDA), and nutrient agar (NA) and larch sapwood meal (LSW). Fig:55 shows that the rate of radial growth was least on NA and best on YEME, PDA. LSW and CMA. The results of an analysis of variance comparing agar media and fungal growth showed significant differences between the treatments after 7, 10, 13, 16 and 19 days' incubation, as shown by the bars for 5% *LSD* in Fig:55.

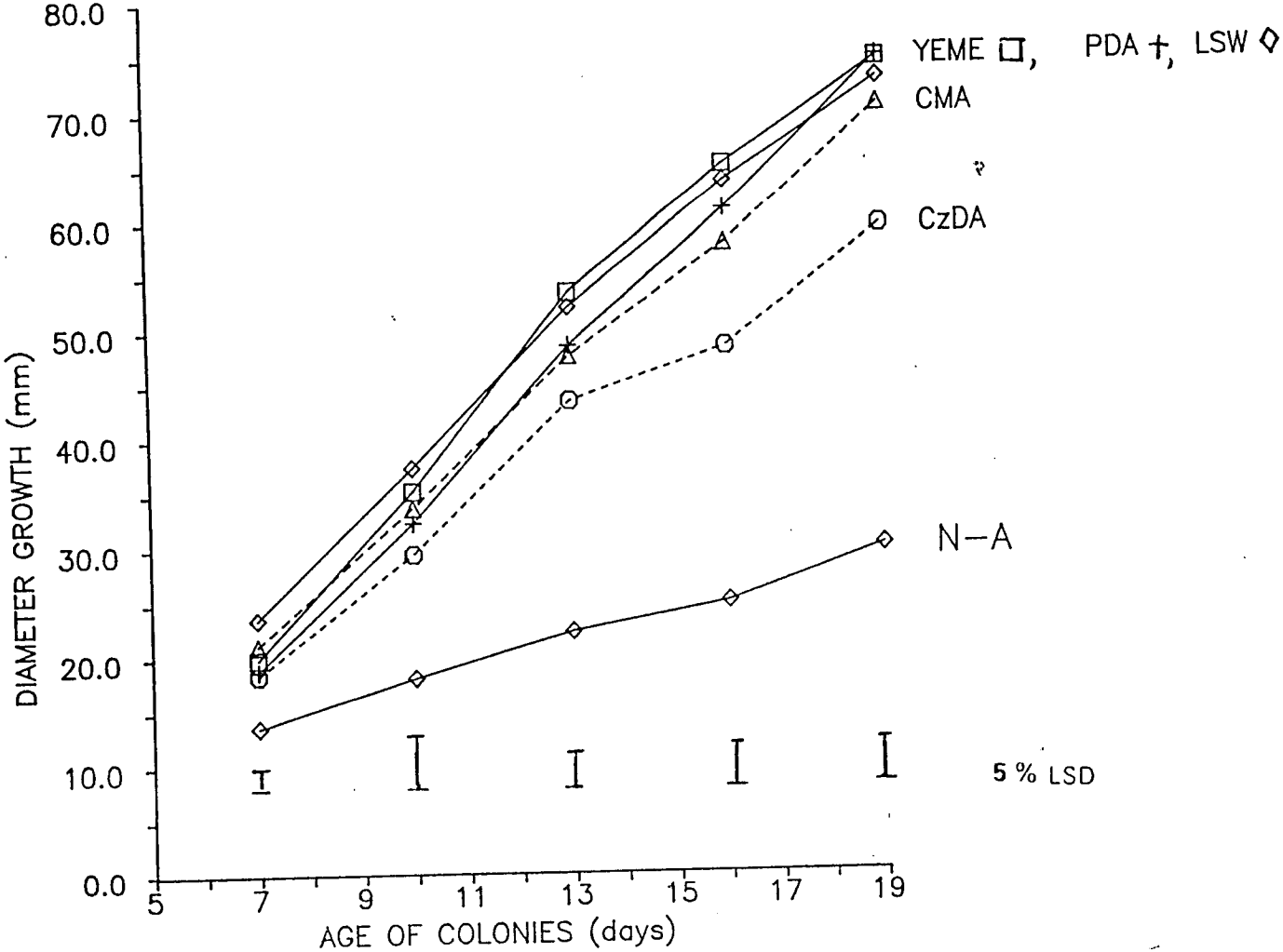


Fig:55 Growth rate of *Ceratocystis piceae* on different agar media and larch sapwood/agar meal at 20°C.

9.2. Effect of larch bark and sapwood

After 3 days incubation, growth of all colonies on larch bark meal (LB) were a dense light grey aerial mycelium. A heavy coremial growth was present. On sapwood meal (LSW), the colonies were grey, radiating submerged hyphae. Coremia were not present. The effect of LB and LSW on radial growth of *C. piceae* did not differ significantly between trees (Fig: 56). However, there were consistent differences between growth on LB and LSW.

9.3. Effect of sapwood and heartwood

Radial growth of *C. piceae* was significantly better on larch sapwood (LSW) than on heartwood/agar (LHW) (Table 52).

Table 52. COMPARISON OF GROWTH OF *Ceratocystis piceae* ON LARCH SAPWOOD (LSW) AND HEARTWOOD/AGAR MEDIA (LHW) AT 20°C.

AGE (days)	MEDIUM	MEAN RADIAL GROWTH(mm)	T-VALUE	DF	PROBABILITY
5	LSW	14.8	3.8	12	0.01
	LHW	11.6			
9	LSW	31.3	2.1	12	0.05
	LHW	27.7			
13	LSW	53.0	6.1	12	0.001
	LHW	40.5			
15	LSW	63.8	9.0	12	0.001
	LHW	51.5			
17	LSW	71.2	12.8	12	0.001
	LHW	57.3			
19	LSW	74.4	12.0	12	0.001
	LHW	62.9			

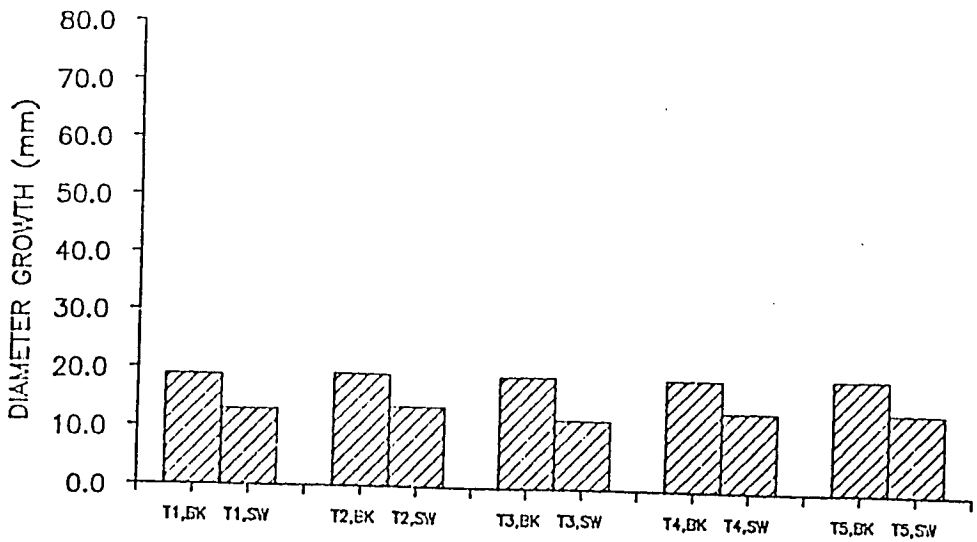


Fig56 Radial growth of *Ceratocystis piceae* on larch bark and sapwood/agar meals after 3 days at 20°C.

9.4. Sapwood and ethanol-soluble carbon and nitrogen fractions in sapwood

After 6 days' incubation of the colonies on the following treatments:- sapwood (LSW), sugar extract (S), amino acid extract (AA), sugars plus amino acids (S+AA), sugars plus organic acids (S+OA) and an extract of sugars, amino and organic acids (S+AA+OA) had produced a hyaline or light grey submerged or appressed mycelial mat. Coremia were not present. After 9 days incubation colonies on LSW had turned to a dark brown or black appressed growth about 20 mm. in diameter around the centre with light grey edges. Coremia were present. Cultures on (S) were light grey submerged hyphae with white-cottony aerial mycelium. Coremia were absent. On AA, colonies had turned into dark brown radiating, submerged hyphae with light grey edges. Coremia were absent. On S+AA, growth was appressed and yellowish-brown. Very few coremia had appeared. On S+OA, colonies had dark brown appressed mycelium in the centre with light grey edges. Few coremia had appeared. Colonies on S+AA+OA, had dark brown, radiating hyphae with light grey edges. Coremia were absent. The pattern of growth remained nearly the same after 26 days incubation when LSW and S+AA+OA attained 74mm of radial growth. No growth at all had occurred on OA and AA+OA. Fig:57 shows the comparisons of the treatments on growth of *C. piceae*.

9.5. Temperature and growth

Figures 58 and 59 show the rate of growth of *C. piceae* on LSW and CzDA at different temperature regimes. Three days after inoculation growth of *C. piceae* was best at 25°C. on both LSW and CzDA. Both LSW and CzDA produced growth at 10 and 15°C. after 4 days' incubation with growth of colonies on LSW and CzDA being better at 15° than at 10°C. On both LSW and CzDA, colonies incubated at 5 and 30°C. showed signs of growth 15 days after inoculation, and growth on LSW and CzDA was better at 30°C. than at 5°C. The optima lay between 15 and 25°C.; the upper limit for growth was

between 30 and 35°C. and the lower limit appeared to be less than 5°C. (Figs 58-59).

9.6. pH and fungal growth

The influence of pH on the growth of *C. piceae* after 19 days on PDA is shown in Fig:60. Linear extension was best on the acidic medium and *C. piceae* showed a wide pH-tolerance in the acid region. The optimum growth apparently lay between pH 3 and 4.

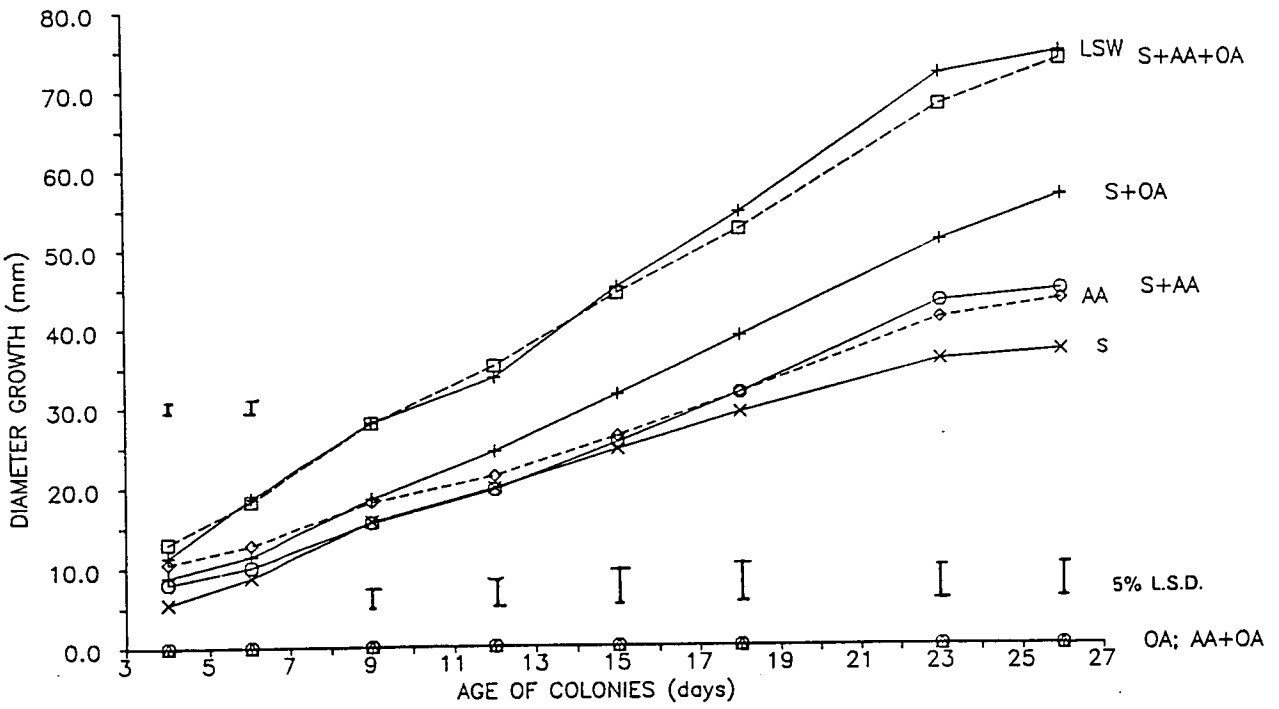


Fig:57 Growth of *Ceratocystis piceae* on larch sapwood and sapwood extracts of sugars, amino acids and organic acids at 20°C.

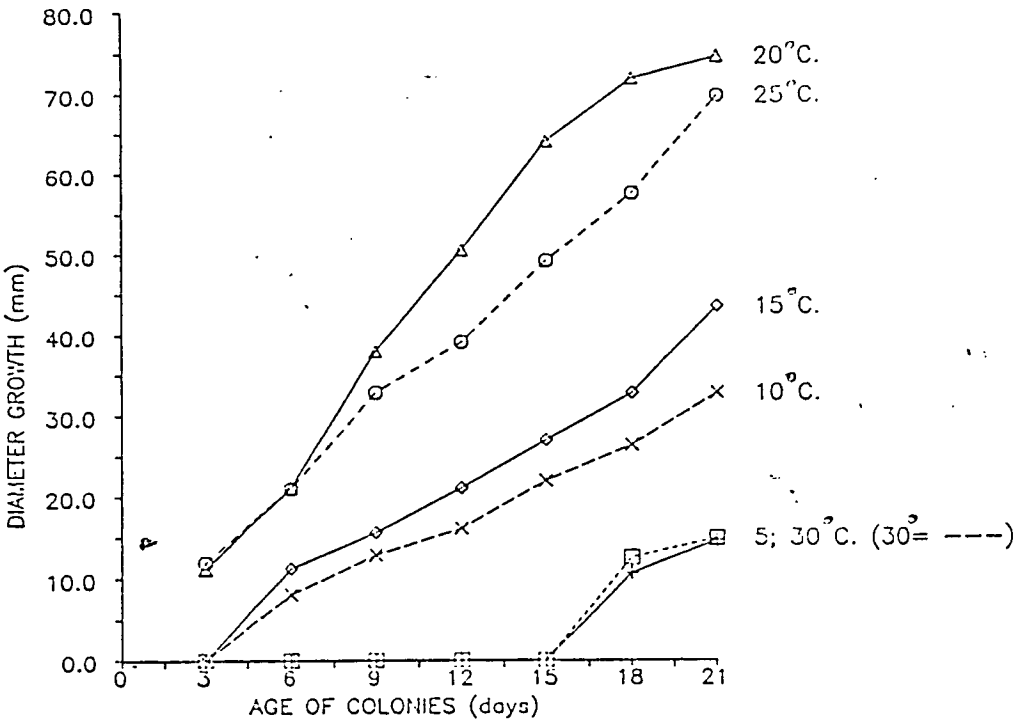


Fig:58 Effect of temperature on growth of *Ceratocystis piceae* on larch sapwood/agar meal.

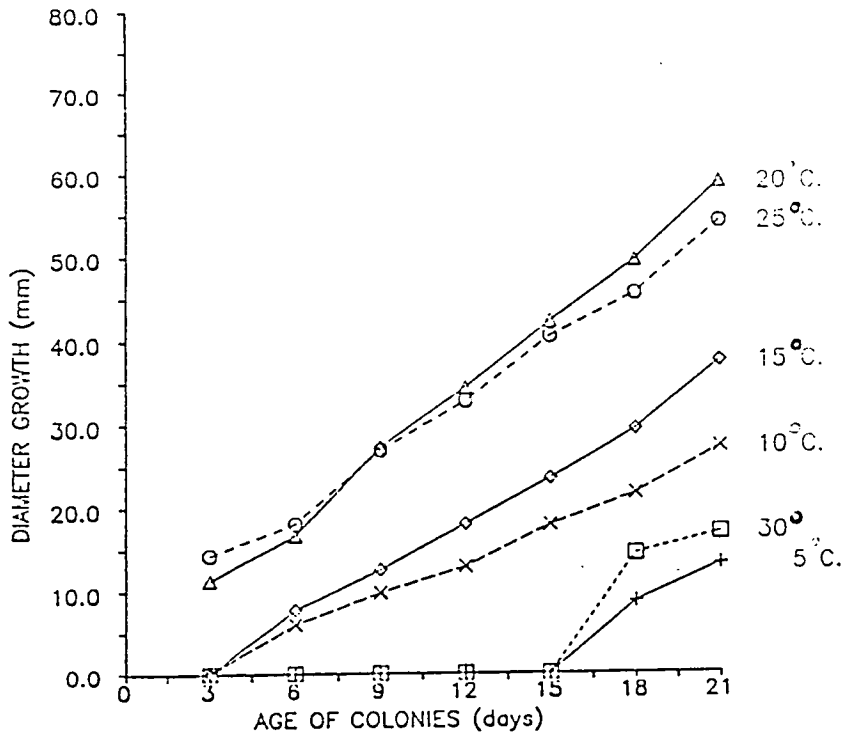


Fig: 59 Effect of temperature on growth of *Ceratocystis piceae* on Czapek dox agar (CzDA) (Modified).

9.7. Carbon nutrition

9.7.1. Effect of glucose, fructose, sucrose and raffinose

The effect of glucose (G), raffinose (R), fructose (F) and sucrose (S) used at 5g/l singly and in different combinations as carbon source on the linear extension of *C. piceae* after 20 days' incubation at 20°C. is shown in Fig:61. The control treatment, with no carbon source, produced the best extension of *C. piceae*, indicating that linear extension *per se* is an inappropriate means of assessing the utilization of different substrates. Unfortunately, the significance of this was not recognized at the time, and the same method was used to compare effects of different carbon and nitrogen sources as shown in Figs 62-70.

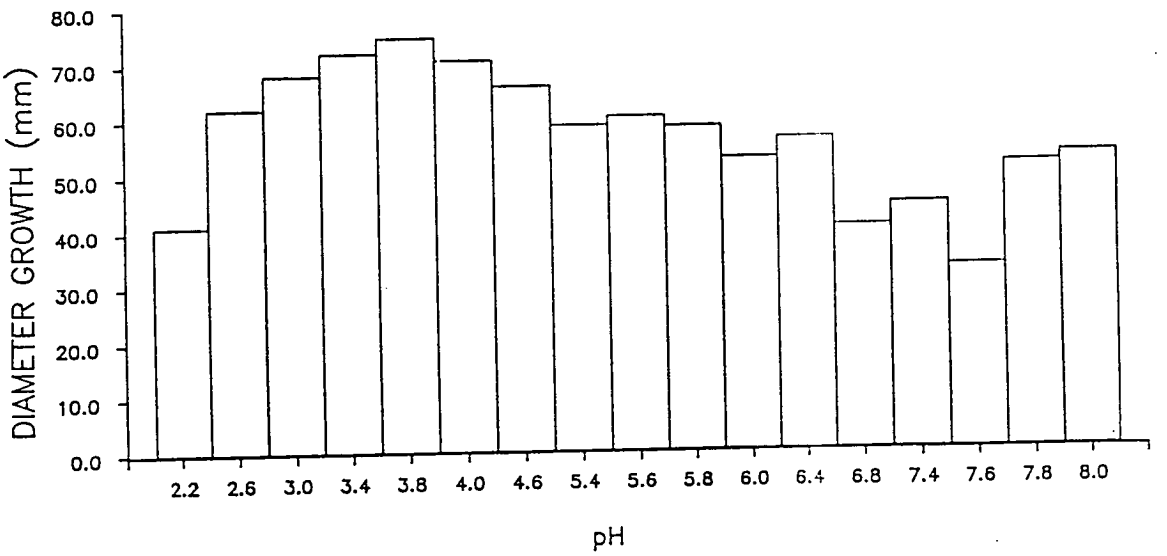


Fig:60 Effect of pH on the linear extension of *Ceratocystis piceae* on PDA after 19 days at 20°C.

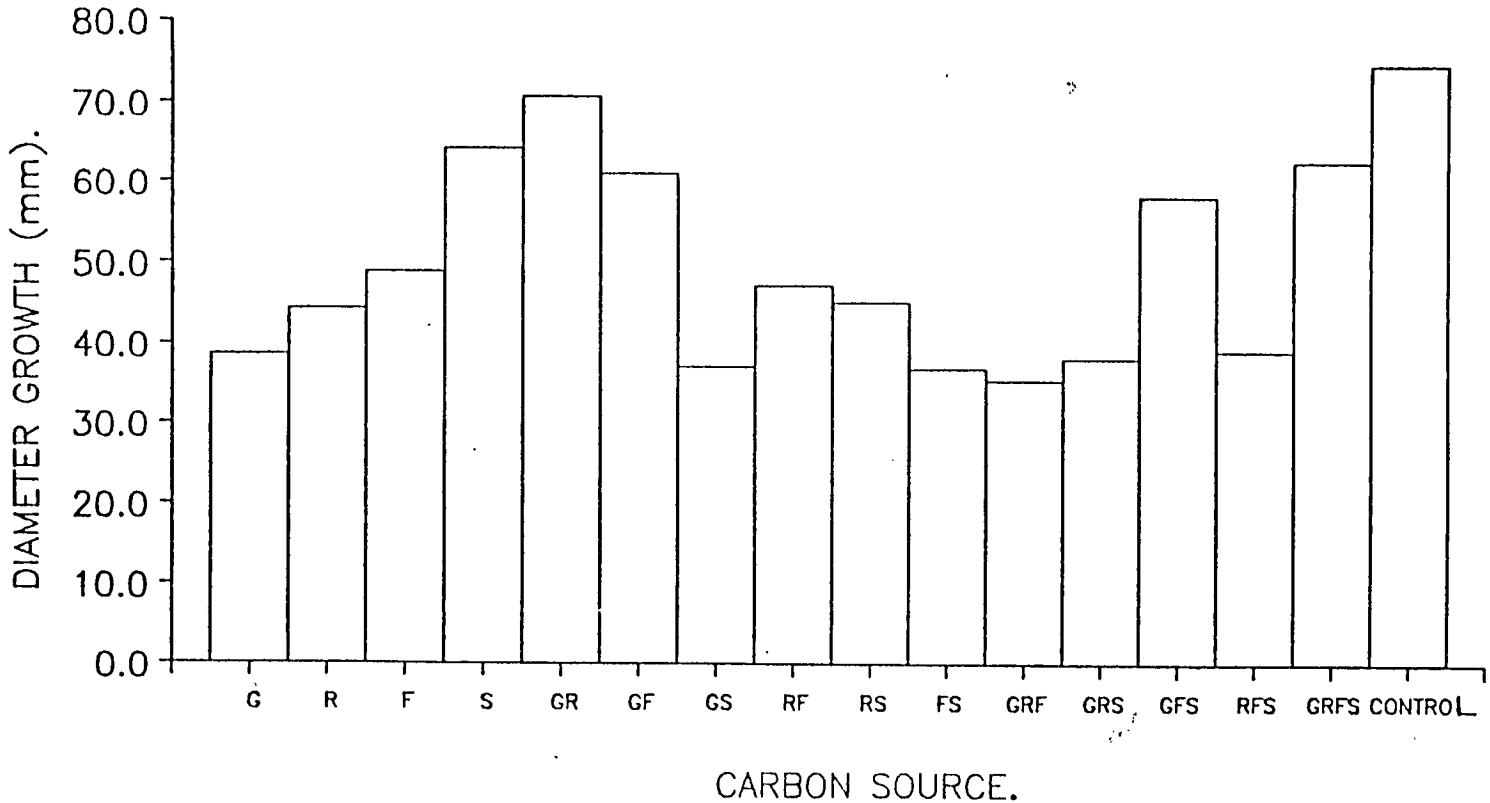


Fig: 61 Radial growth of *Ceratocystis piceae* on 15 carbon sources (individual sugars and sugars in combination) plus a control (no sugar) after 20 days at 20 °C. (ammonium tartrate=nitrogen source).

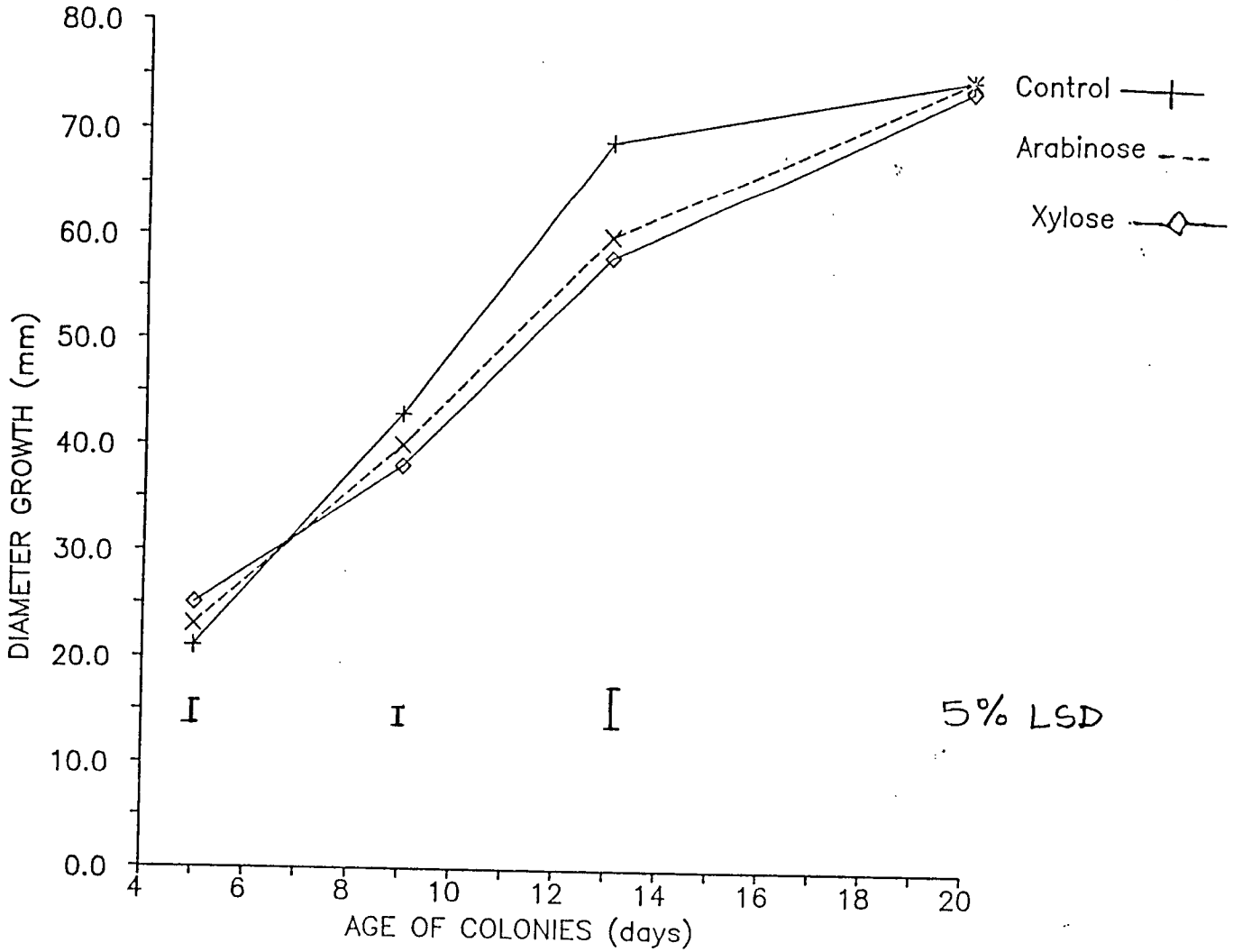


Fig:62 Linear extension rate of *Ceratocystis piceae* on different sources of carbon (ammonium tartrate=nitrogen source); means of 4 replicate agar plates.

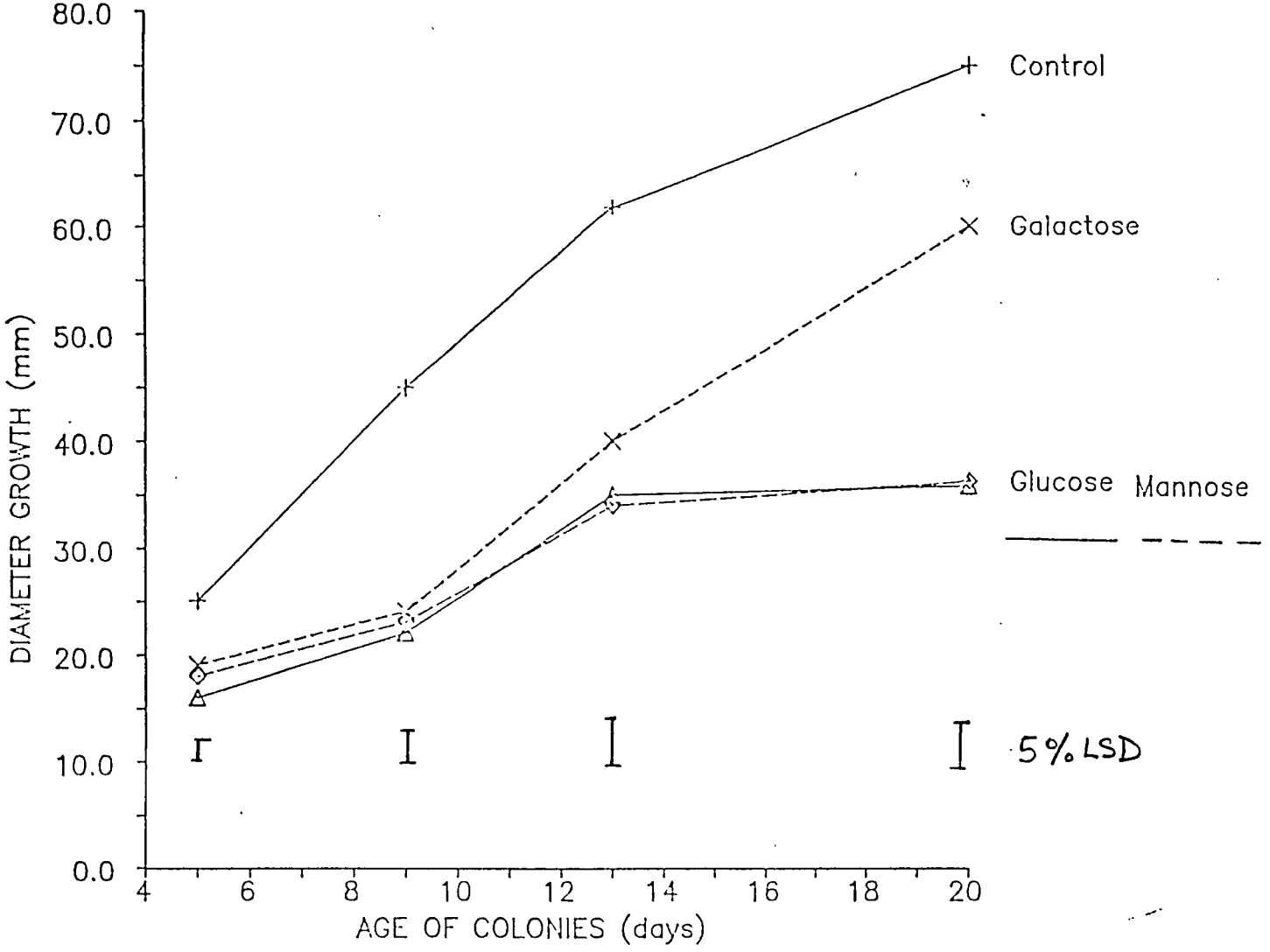


Fig:63 Linear extension rate of *Ceratocystis piceae* on different sources of carbon (ammonium tartrate=nitrogen source); means of 4 replicate agar plates.

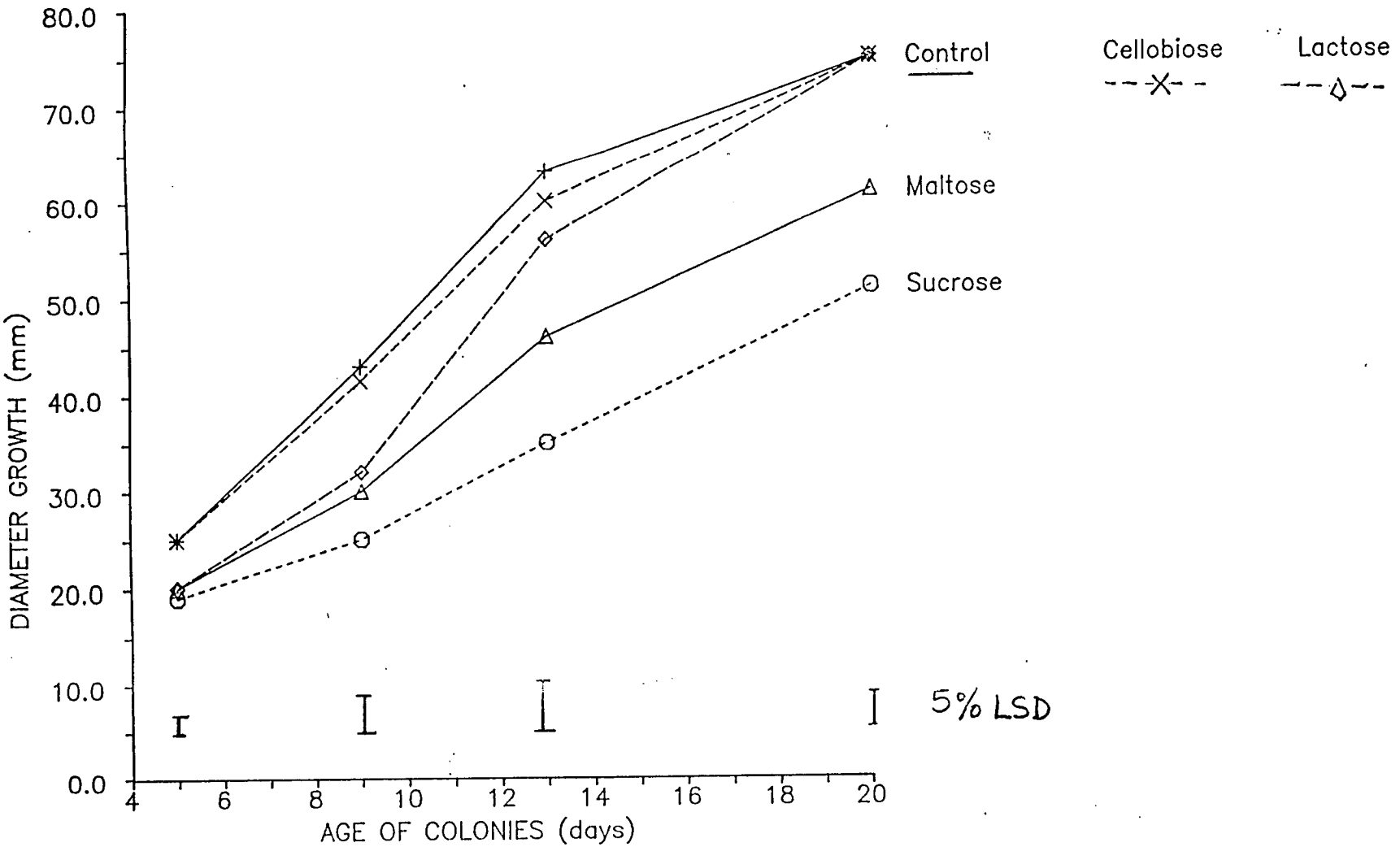


Fig:64 Linear extension rate of *Ceratocystis piceae* on different sources of carbon (ammonium tartrate=nitrogen source); means of 4 replicate agar plates.

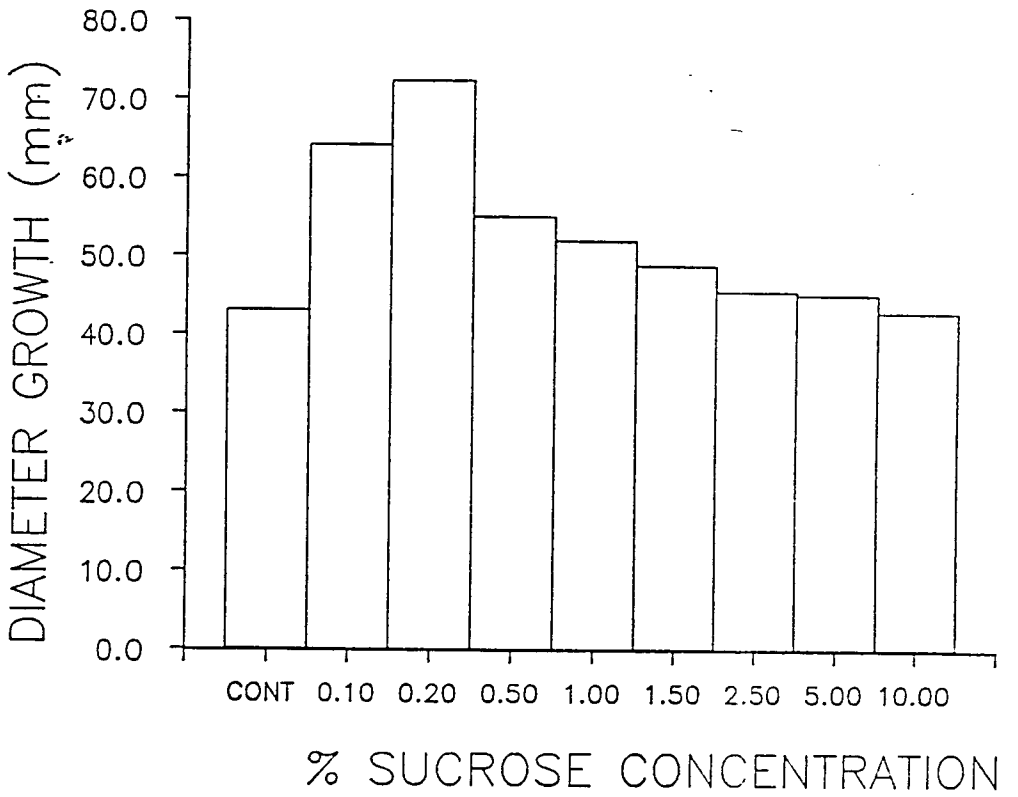


Fig:65 Linear extension of *Ceratocystis piceae* on different levels of sucrose with ammonium tartrate as nitrogen source, after 19 days at 20°C.; means of 6 replicate agar plates.

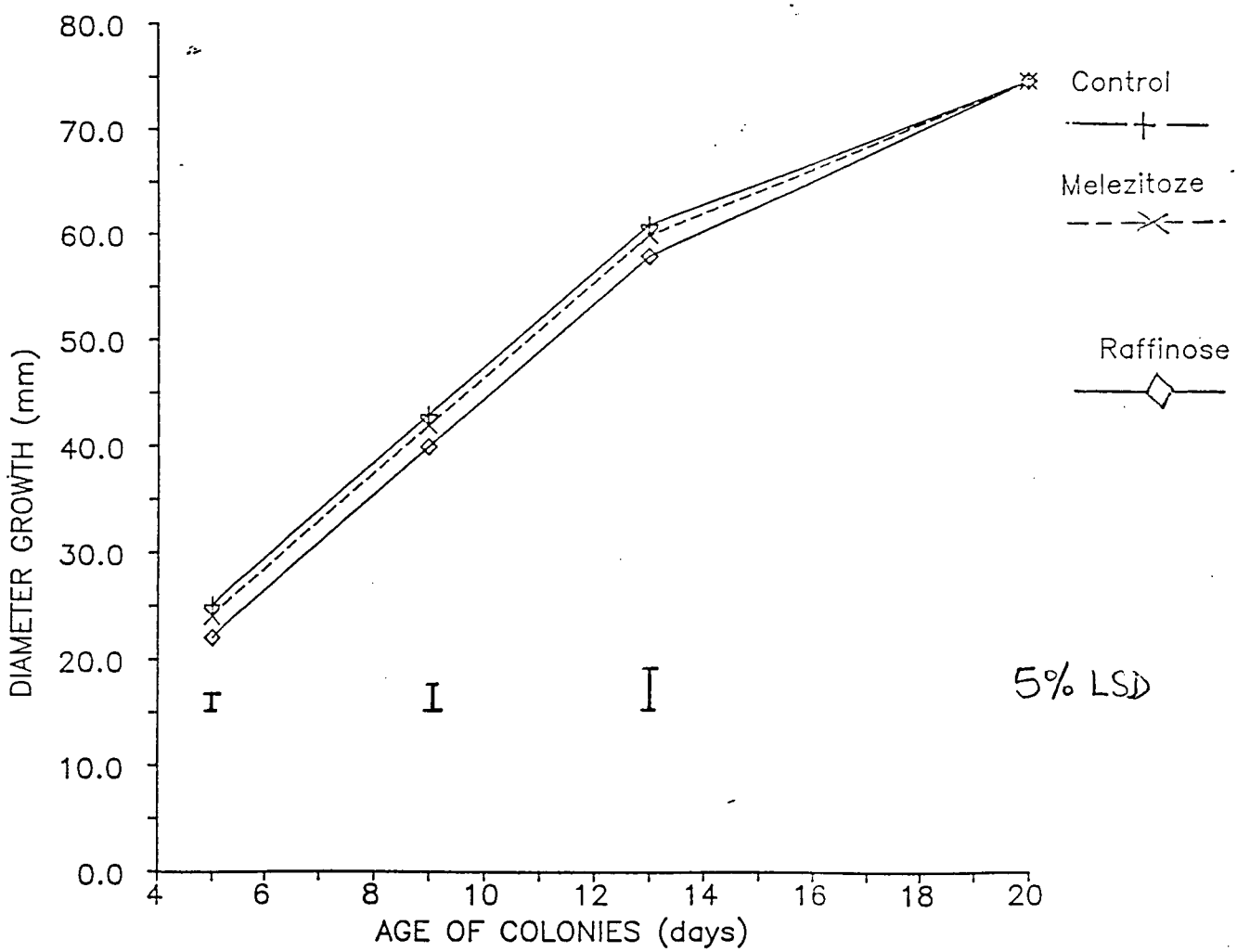


Fig:66 Linear extension rate of *Ceratocystis piceae* on different sources of carbon (ammonium tartrate=nitrogen source); means of 4 replicate agar plates.

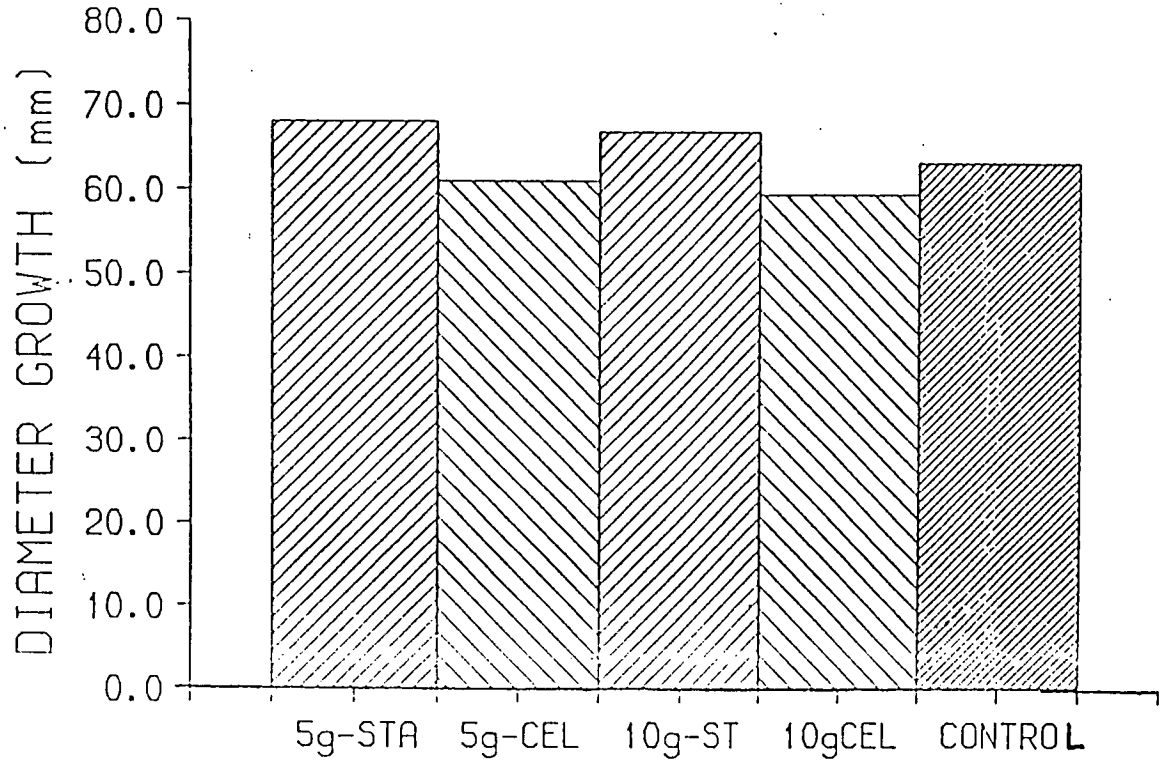


Fig:67 Linear extension rate of *Ceratocystis piceae* on different levels of starch and cellulose (5 or 10g/l.) with ammonium tartrate as nitrogen source, after 15 days at 20 °C. .; means of 4 replicate agar plates.

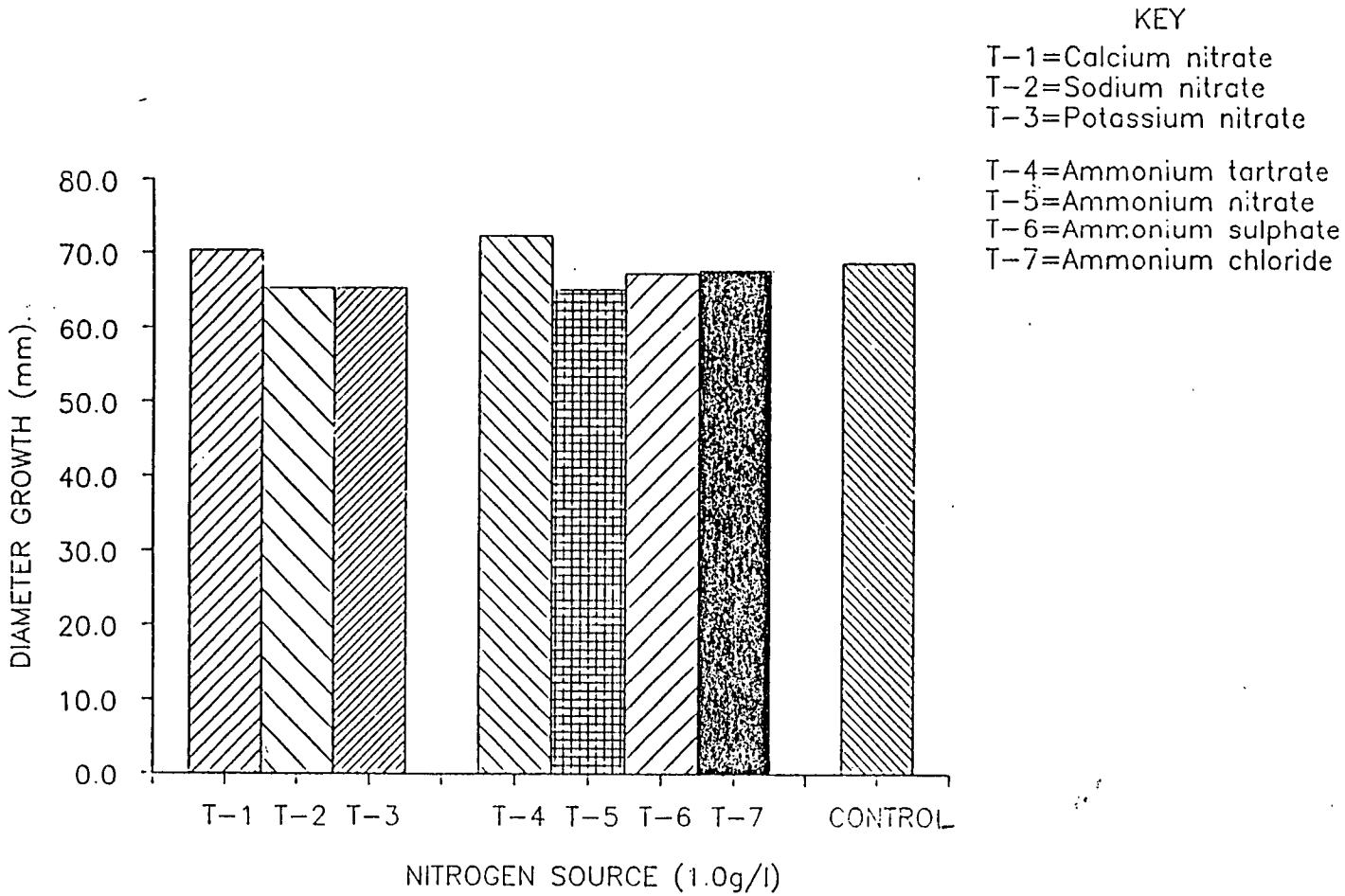


Fig:68 Linear extension of *Ceratocystis piceae* on media containing different nitrate and ammonium nitrogen after 19 days at 20°C. (glucose=carbon source); means of 6 replicate agar plates.

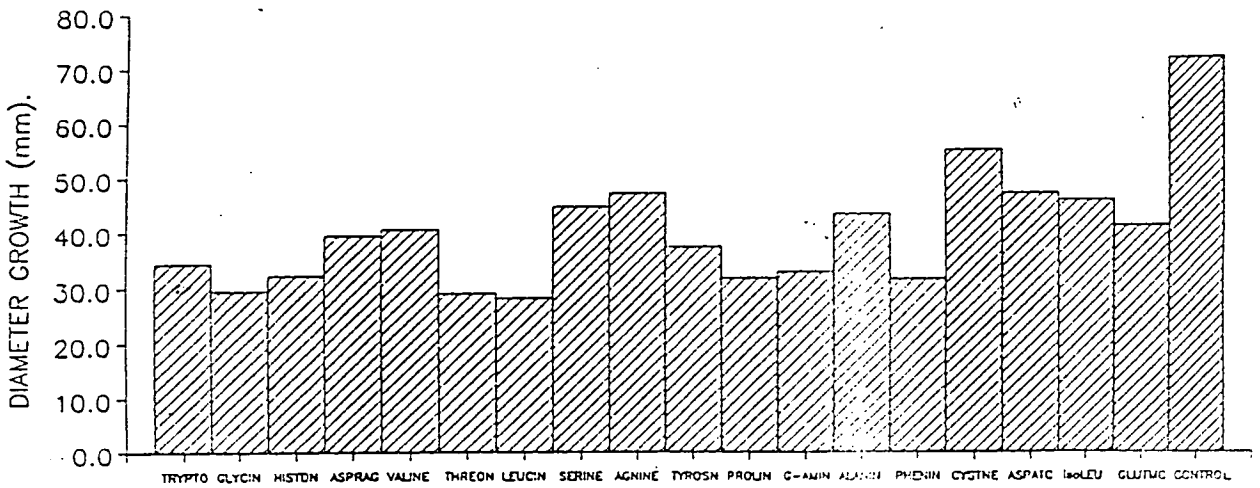


Fig:69 Linear extension of *Ceratocystis piceae* on 18 nitrogen sources (amino acids at 0.1g/l.) after 15 days at 20°C. (glucose= carbon source, 10.0g/l.); means of 4 replicate agar plates.

TRYPTO = Tryptophane
GLYCIN = Glycine
HISTDN = Histidine
ASPRAG = Asparagine
VALINE = Valine
THREON = Threonine
LEUCIN = Leucine
SERINE = Serine
AGNINE = Arginine
TYROSN = Tyrosine

PROLIN = Proline
G-AMIN = gamma-Amino butyric acid
ALANIN = Alanine
PHENIN = Phenylalanine
CYSTNE = Cystine
ASPATC = Aspartic acid
isoLEU = *iso*-Leucine
GLUTMC = Glutamic acid
CONTROL = Control (no nitrogen).

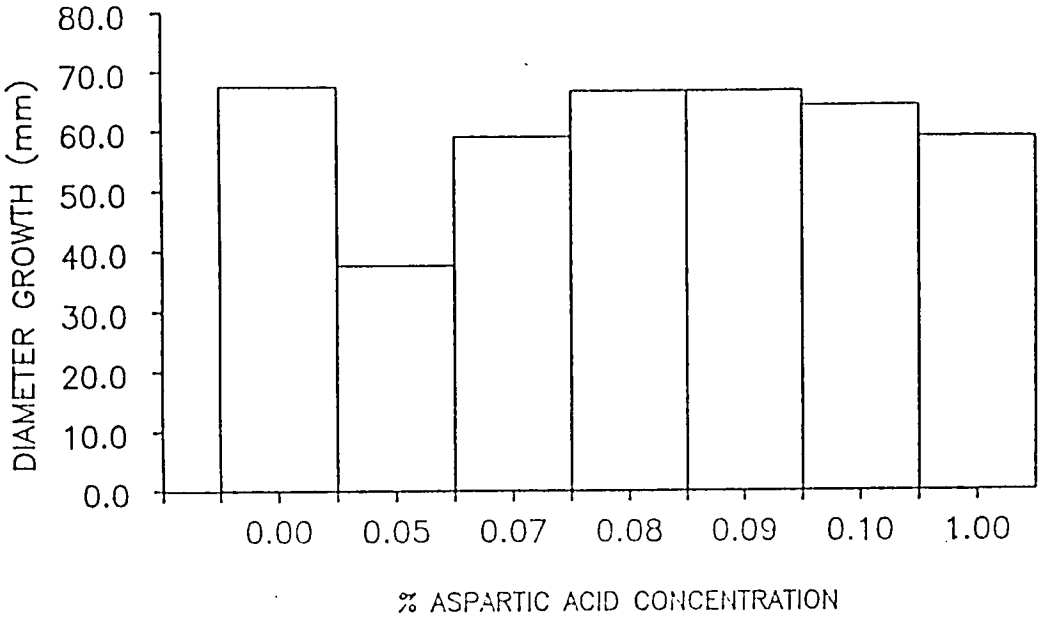


Fig:70 Linear extension of *Ceratocystis piceae* on different levels of aspartic acid with sucrose as carbon source, after 20 days at 20°C.; means of 7 replicate agar plates.

9.8. Spore production by *C. piceae*

9.8.1. Effect of agar media and sapwod/agar

Colonies of *C. piceae* on YEME consisted of dense white-yellowish cottony aerial mycelium on light grey submerged mycelia with hyaline edges. On the upper side, 5 distinct zones were observed as follows: (1) the centre was covered with aerial mycelium. Coremia appeared 7 days after inoculation; (2) white-yellowish mycelium. Coremia were not present; (3) yellow appressed mycelium and was mostly slimy. Coremia were absent; (4) grey to light brown zone. Coremia were absent; (5) hyaline edge. Coremia were absent. Zonation was distinct when the colony was viewed from the underside.

Colonies on PDA were mostly yellowish with appressed mycelium. Colony edges were hyaline to light grey and zonation was not distinct. Coremia appeared after 7 days' incubation, and covered the whole hyphal surface.

Growth on CMA was hyaline to light grey with submerged hyphae which turned green around the centre. Aerial mycelium was scanty. Zonation was very slight. Very few coremia appeared after 10 days' incubation, and they were restricted to the centre.

On CzDA aerial growth was initially hyaline to light grey, but turned yellowish-green in older cultures. Numerous coremia appeared seven days after inoculation. Zonation was distinct: (1) the centre was hyaline to light grey and with cottony mycelium. Coremia were absent; (2) the next zone was yellow turning light brown. Abundant coremia were present; (3) a white-cottony zone. Coremia were not present; (4) a light grey zone. Few coremia were present and; (5) a hyaline zone. Coremia were not present. Zonation was distinct when the colony was viewed from the underside.

Colonies on LSW did not show distinct zones. However, the centre had a dark brown discolouration, followed by a wide band of light grey submerged hyphae which bore distinct symmetric bands of abundant coremia. Coremia appeared after 4 days' incubation.

Growth on NA was mostly appressed, hyaline to light grey mycelium, with numerous tiny water droplets around the centre. There were 3 distinct zones; (1) the centre was initially hyaline, then turned yellow; (2) white-cottony appressed growth; (3) the edge was hyaline or yellowish. The underside was a single yellowish-light brown layer. There were no coremia on any cultures after 19 days' incubation.

9.8.2. Effect of larch bark and sapwood

After 3 days incubation, growth of all colonies on larch bark meal (LB) were a dense light grey aerial mycelium. Heavy coremial development was present. On sapwood meal (LSW), the colonies were grey, radiating submerged hyphae. Coremia were not present.

9.8.3. Effect of sapwood and heartwood

Five days after inoculation all colonies on LSW and LHW produced a thin layer of almost transparent submerged hyphae. 19 days after inoculation, colonies on LSW were appressed, white-dark grey and cottony. Zonation was more distinct on colonies on LHW: the centres were light grey and the 10 mm wide edges were hyaline.

Four days after inoculation colonies on LSW produced coremia arranged in concentric circles. Colonies on LHW produced coremia after 9 days' incubation. After 13 days, it became evident that colonies on LSW produced many more coremia with larger heads than colonies on LHW. However, the concentric circles of coremia were produced in a more regular pattern on LHW than LSW, and the whole hyphal surface on LSW was covered with coremia,

while on LHW only the centres had coremia.

9.8.4. Effect of sapwood and ethanol-soluble carbon and nitrogen fractions in sapwood

After 6 days' incubation of the colonies on the following treatments:- sapwood (LSW), sugar extract (S), amino acid extract (AA), sugars plus amino acids (S+AA), sugars plus organic acids (S+OA) and an extract of sugars, amino and organic acids (S+AA+OA) had produced a hyaline or light grey submerged or appressed mycelial mat. Coremia were not present. After 9 days incubation colonies on LSW had turned to a dark brown or black appressed growth about 20mm in diameter around the centre with light grey edges. Coremia were present. Cultures on (S) were light grey submerged hyphae with white-cottony aerial mycelium. Coremia were absent. On AA, colonies had turned into dark brown radiating, submerged hyphae with light grey edges. Coremia were absent. On S+AA, growth was appressed and yellowish-brown. Very few coremia had appeared. On S+OA, colonies had dark brown appressed mycelium in the centre with light grey edges. Few coremia had appeared. Colonies on S+AA+OA, had dark brown, radiating hyphae with light grey edges. Coremia were absent. The pattern of growth remained nearly the same after 26 days incubation when LSW and S+AA+OA attained 74mm of radial growth. No growth at all had occurred on OA and AA+OA.

9.8.5. Effect of temperature

Temperature affects the cultural characteristics of *C. piceae*. At 5, 10, 15 and 30°C. growth was mostly characterised by hyaline to light grey submerged or aerial hyphae. Coremia were absent. Colonies on LSW kept at 20 and 25°C. were darkly stained, with the stain being darker at 25 than 20°C. Colonies at 20°C. produced coremia which were arranged in concentric bands, colonies at 25°C. produced coremia which were randomnly distributed.

9.8.6. Effect of pH

After 5 days' incubation, growth at pH's 2.2–4.0, was characterised by light grey, sparse white-cottony aerial mycelium with water droplets. Coremia were absent. Colonies at pH 4.6 had brown centres with light grey, about 5mm wide edges. Numerous coremia were present. At pH's 5.4–5.8, colonies had darker centres than those at pH 4.6. Coremia were absent at pH 5.4–5.8. At pH's 6.0–6.4 the brown stain was considerably reduced, and most colonies were light grey. Coremia were absent. At pH's 6.8–8.0, the cultures had white-cottony aerial mycelium. Coremia were absent.

After 9 days incubation there was no change in the growth pattern of *C. piceae* at pH's 2.2–4.0, and at pH 4.6. Coremia had appeared at pH's 5.4–5.8, and their density decreased from pH's 5.4–5.8. Colonies at pH's 6.0–6.4 had become darker. Coremia were absent. There was no visible change in the growth at pH's 6.8–8.0. Coremia appeared on colonies at pH's 2.2–4.0 and 6.0 after 11 days' incubation.

14 days after inoculation colonies at pH's 5.4–5.8 were much darker and had a higher density of coremia than those at pH 4.6. After 19 days incubation, colonies at 5.6 had the highest density of coremia. From pH 5.6, the density of coremia decreased with the decrease in the pH down to pH 2.2, and also decreased with the increase in the pH up to pH 8.0. Therefore, *C. piceae* seems to have a symmetric coremia production curve around an optimum of about pH 5.6, which contrasts with the results for radial extension (Fig: 60) but is probably a better indication of the pH optimum for growth of the fungus.

9.8.7. Effect of carbon sources

Five days after inoculation all colonies were mixtures of yellow, green, grey and brown colours. Coremia were absent on all colonies. Colonies on raffinose (R) and fructose (F) produced coremia after 9 days' incubation.

Colonies on raffinose had small young coremia, while those with fructose had abundant and fully grown coremia. After 12 days' incubation coremia appeared, in increasing order on colonies on (1) glucose, (2) a mixture of glucose, raffinose and fructose (GRF), glucose, raffinose and sucrose (GRS), glucose and sucrose (GS); (3) fructose and sucrose (FS); (4) raffinose, fructose and sucrose (RFS), raffinose and fructose (RF); (5) raffinose and sucrose (RS); (6) raffinose and (7) fructose. After 19 days' incubation all colonies had produced coremia. Among the single carbon sources used, colonies on sucrose produced the smallest number of coremia.

9.8.7.1. Aldopentoses

After 15 days' incubation, colonies with xylose and arabinose had produced a light yellowish-green mycelial mat in the centre, about 10 mm diameter, with smooth light grey or almost transparent edges. Only colonies with arabinose had produced coremia. Colonies with xylose produced coremia after 9 days' incubation. After 20 days' incubation, colonies with arabinose had produced more coremia than those with xylose. Colonies with xylose had turned yellowish and/or greenish-brown with a thick, dark brown mycelial mat in the centre (40-54.5 mm diameter) with light grey edges. Colonies with arabinose had a sparse, white-cottony mycelial mat in the centre with light to dark brown edges.

9.8.7.2. Aldohexoses

After 5 days' incubation all colonies had a central (10-15 mm diameter) yellowish-green-cottony mycelial mat, with hyaline or light grey edges. Coremia were not present on the colonies. However, colonies with glucose and mannose produced coremia after 9 days' incubation, and colonies with galactose produced coremia after 13 days' incubation. After 20 days' incubation, colonies with glucose and mannose had many more coremia than those with galactose.

9.8.7.3. Disaccharides

Five days after inoculation onto plates containing lactose, sucrose, maltose and cellobiose *C. piceae* had produced a central (10–15 mm diameter) light yellowish-green mycelial mat, with hyaline or light grey edges. Only colonies with cellobiose had failed to produce coremia. However, colonies with cellobiose produced coremia after 9 days' incubation. After 20 days' incubation, colonies with lactose had the largest number of coremia.

9.8.7.4. Sucrose

The control treatment had a thin, submerged hyphal growth, with smooth edges. Very few coremia were present. Colonies with 0.1 per cent sucrose had a denser aerial growth than those on the control treatment, and colonies on 0.10 per cent were yellowish-brown with patches of green colour. Colonies on 0.10 per cent had many more coremia than those on the control treatment. Colonies on 0.20 per cent had a denser white-cottony mycelial mat, and had many more coremia than those on 0.10, 0.50 and 1.00 per cent. The density of mycelial growth, and production of coremia, decreased from colonies on 0.50 to those on 10.0 per cent sucrose concentration. Both mycelium and coremia production increased from 0.00 per cent to 0.10 percent, peaked at 0.20 per cent, and then decreased towards the lowest level at 10.0 per cent.

9.8.7.5. Trisaccharides

Five days after inoculation, colonies with raffinose and melezitose were yellowish-green in the centre (10–15 mm diameter) with slightly slimy light grey edges. Only colonies with raffinose had produced a dense growth of coremia. Colonies with melezitose produced coremia 9 days after incubation. Twenty days after inoculation, colonies with raffinose had turned into a yellowish-brown and/or grey mycelial mat, and the light grey edges had disappeared. Colonies with melezitose had produced a thick mycelial mat in the centre (40–45 mm diameter) with smooth light grey edges. Colonies with

raffinose produced many more coremia than those with melezitose.

9.8.7.6. Cellulose and starch

Nine days after inoculation the growth of *C. piceae* on cellulose was appressed, light grey to yellowish-green mycelium, while on starch it was a light grey dense-cottony mycelial mat with radiating submerged hyphae. Only colonies on cellulose at 10g/l produced numerous coremia, and colonies on the control treatment had few coremia.

9.8.8. Effect of amino acids

The 18 amino acids were classified into five categories on the basis of their effect on the radial growth and spore production in *C. piceae*. Group 1: leucine, glycine, proline, phenylalanine, histidine, tyrosine and asparagine had after 10 days' incubation, produced a thick yellowish-green mycelial mat with uneven edges. Coremia were not present. Colonies with glycine, proline and phenylalanine had a darker yellow stain but with a less darker green stain than colonies with leucine, histidine, tyrosine and asparagine. Colonies with histidine, tyrosine and asparagine produced coremia 15 days after inoculation, and colonies with leucine, glycine, proline and phenylalanine failed completely to produce coremia. Group 2: colonies with tryptophane and those on the control treatment produced a light greenish-brown submerged hyphal layer with smooth edges. A few small coremia were present. Group 3: colonies with threonine and valine had produced a thick yellowish-green aerial mycelial mat over a dark green submerged hyphae, with about 5mm wide light grey edges. Colonies with threonine and valine produced many more coremia than colonies with amino acids listed in groups 1 and 2. Group 4: colonies with gamma-amino butyric acid and alanine produced a thick yellow-cottony aerial mycelial mat over a green-black submerged layer. Colonies with alanine had a thinner aerial mycelial mat, with a darker yellow stain than colonies with gamma-amino butyric acid. Colonies with gamma-amino butyric acid produced

more coremia than those with alanine. Group 5: colonies with glutamic acid, iso-leucine, aspartic acid, arginine, serine and cystine had a dark brown appressed mycelial mat with smooth edges. Abundant large coremia were present on all colonies, and in order of decreasing importance, cystine, serine and arginine had more coremia than other amino acids used.

In each of the five groups of amino acids listed above, the amino acid which produced the best mycelial growth also produced the best coremia growth. There seemed to be a direct relationship between radial and coremia growth in *C. piceae*.

CHAPTER 10

CHAPTER 10

DISCUSSION

In common with other Coleoptera, *Hylecoetus dermestoides* exhibits sexual dimorphism; morphological features such as size, colour and maxillary palpi being different in the two sexes. Sexual dimorphism, and the morphological variation within male *Hylecoetus* was previously reported by Fergusson (1920) and Thomsen *et al* . (1949). However, there are other distinct characteristics of males which need clarification. In the Scottish race of *Hylecoetus*, both all-black and yellowish brown adult males occur together. In the present study, a total of 196 males were examined, 175 of which were all-black and 21 yellowish brown giving an over-all ratio of 8.3:1. The proportion of all-black:yellowish brown males has not been previously quantified. However, previous British records indicate the male as being yellowish brown (sometimes all-black) (Fergusson, 1920; Chinery, 1982). Thomsen *et al* . (1949) reported that in the Danish race of *Hylecoetus* as a rule the male is all-black (sometimes brown). This study confirms the existence of two male adult *Hylecoetus* forms being in Scotland, but the reason for the two colour forms cannot be explained.

Although there are no previous records of the sex ratio in the Scottish race of *Hylecoetus*, the present study shows approximately a 1:1 sex ratio. This result does not agree with observations by Thomsen *et al* . (1949) and Kurir (1972) who gave a sex ratio of ♀1:2.29♂ (n=56) and ♀1:1.98♂, indicating that twice as many males as females can be found in Denmark and Austria respectively. Geographical variation may be an explanation for the difference between the sex ratio obtained for Scotland and those from the Continent.

Considerable size variation exists in adult *Hylecoetus*. Females are

significantly larger than males; females being 10.3 to 16.4 mm. and males 8.2 to 12.7 mm. long respectively. These results are in good agreement with those by Fergusson (1920) who found that females were 9 to 16 mm. and males 6 to 13 mm. long. In addition, females have longer elytra than males, confirming the earlier results of Thomsen *et al* . (1949) in Denmark. The ratio of elytron length to total body length is about 80% in both female and male *Hylecoetus*. There is a marked difference in size between pupae and adult *Hylecoetus*. Adult females are 24% smaller than female pupae, whereas male adults are 9% smaller than male pupae. This difference in pupae and adult *Hylecoetus* and indeed in other Coleoptera has not been given due consideration. Fergusson (1920) speculated that the size of *Hylecoetus* was influenced by nutrition, with underfeeding resulting in some dwarf forms. Quantitative nutritional data on *Hylecoetus* are lacking.

During the duration of the present study (1983-86), adult *Hylecoetus* began emerging in April and flew from the end of April to the end of June. This contrasts with Fergusson's earlier (1920) study where *Hylecoetus* were observed to appear in May-June and to fly in June-July.

Pairing in *Hylecoetus* has been previously observed under natural conditions by Fergusson (1920) and in the laboratory by Thomsen *et al* . (1949) and Kurir (1972). In the present study mating was observed in the field and the results show that the diurnal mating pattern, which has not been previously reported, is similar to the diurnal flight pattern, and both activities coincide and have similar peaks.

The present study showed that adult *Hylecoetus* are short lived (1-3 days), and agrees well with earlier findings by Thomsen *et al* . (1949), Thomsen (1950), Kurir (1972) who indicated that the longevity of emerged adults ranged from 1-4 days. Short lived adult Coleoptera usually live in quite different

habitats, and on quite different food from their larvae, and many do not normally take protein food (Crowson, 1981). Feeding by adult *Hylecoetus* has not been documented in this study.

Under natural conditions oviposition by female *Hylecoetus* occurs throughout the flight season, and most oviposition occurs during peaks of flight. Fergusson (1920) reported that oviposition occurs throughout the flight season. Part of the female *Hylecoetus* population (total 7) laid eggs on the day they emerged, while the rest (total 24) laid eggs on the second day after emergence, confirming in part Thomsen *et al* . (1949) who reported that female *Hylecoetus* lay eggs on the day they emerge. Eggs are laid in batches, with batches of eggs per female ranging from 1 to 3; first batch, 4–45; second batch, 12–29; and third batch, 5–20 eggs respectively. There is no significant difference in the number of eggs per batch; each female laid a total of 7–61 eggs. Female *Hylecoetus* kept on agar at 20°C. produced only one batch of eggs each, with a mean of 18 eggs per batch per female respectively. Numbers of eggs laid per batch between natural and laboratory populations did not differ significantly. Females on agar laid eggs 1–2 days after emergence. It is not clear why laboratory cultures had only one batch of eggs per female. Fergusson (1920) reported that eggs are laid singly, whereas Thomsen (1950) reported that eggs were laid either singly or a few together. This contradicts the observations by Kurir (1972) who reported that eggs were laid in heaps, with 4–91 eggs per heap; single or pairs of eggs being seldom; and each female *Hylecoetus* produced a total of 99–146 eggs. The present findings agree with observations by Kurir (1972). However, in the Austrian race of *Hylecoetus* the female lays more eggs (99–146 eggs per female) than the female in the Scottish race (7–61 eggs per female). In 1984 more eggs were laid on warmer days than on cooler days. This indicates a possible influence of temperature on oviposition by *Hylecoetus*. *H. dermestoides* flies earlier in Austria than in

Scotland, and by inference (cf. Bakke, 1968) flight temperature threshold for *Hylecoetus* may be lower in Austria than in Scotland. Therefore, the number of eggs laid by female *Hylecoetus* of the Austrian race should be less than those laid by females in the Scottish race. However, the smaller number of eggs laid by the Scottish race may be attributed to sampling method used. In this study egg counts were made on females caught in flight, and it was not possible to determine whether or not any eggs had been laid before the beetles were captured. Dick (1938) reported that in other Coleoptera, *Tenebrio* and *Tribolium*, the number of eggs laid is influenced by temperature.

In this study laboratory reared virgin female *Hylecoetus* laid fewer eggs than mated females from natural populations, and all the eggs laid by virgin females failed to hatch. The virgin females used for oviposition in the present study originated from larvae which were reared on agar at 20°C. for as long as 200 or more days, during which periodically the larvae were surface-sterilised and the agar renewed. This rearing technique produced ectosymbiotic fungi-free *Hylecoetus*. Lack of copulation and ectosymbiotes seems to have influenced the number and viability of eggs laid by virgin female *Hylecoetus*. Dick (1938) reported that copulation, besides supplying spermatozoa, has a stimulating effect on the rate of ovipostion in *Tenebrio confusum* Duv (Tenebrionidae). Norris (1972) noted that: (1) when ectosymbiotic fungi-free, virgin female *Xyleborus ferrugineus* (Scolytidae) reared on synthetic diets with antibiotics, the number of ovipositing females was significanly reduced as compared to those on antibiotic-free diet. The number of progeny/female in the diets containing antibiotics was also significantly reduced. The nucleus of oocyte yolk in females reared on antibiotic-containing diet remained static i.e. none matured. When antibiotic-treated non-laying females were subsequently transferred to diet with *Staphylococcus* they unfailingly began to lay eggs within 4-5 days. This indicates that some chemical entity or entities produced

by the *Staphylococcus* allow oocyte maturation. Normally all maturing oocytes and laid eggs contain this coccus; (2) oocytes do not develop beyond the primary oocyte stage in the absence of the bacterial symbiote. Thus, sexual as well as asexual reproduction is primarily dependent upon the *Staphylococcus*. In the presence of an adequate population of the bacterium, embryonic development is initiated while the eggs are still enclosed in the follicular walls of the ovarioles. Therefore, the usual sperm role of mature-oocyte activation in sexual reproduction is indeed assumed by the bacterial symbiote. The remaining major role of the sperm thus seems to be the creation of the diploid progeny state which yields females. French and Roeper (1975) reported that oocyte development and oviposition in the ambrosia beetle *Xyleborus dispar* (Scolytidae) only occurred after the female had fed on the ambrosial form of its ambrosia fungus *Ambrosiella hartigii* Batra. Whether the microorganisms associated with *H. dermestoides*, e.g., *Ceratocystis piceae*, etc., control the fertility of females; whether the periodic surface sterilizing of larvae and renewal of agar really produced ectosymbiotic fungi-free *Hylecoetus* and whether the absence of sperm were all responsible for the production of non-viable eggs by virgin females is not known. Parthenogenesis in Lymexylids is not possible, as indicated by Dick (1938) who stated that parthenogenesis in Coleoptera is confined to the Curculionidae, and even there, it is rare. Of the species he studied, *Tenebrio molitor* L., *Tribolium confusum* (Tenebrionidae); *Sitodrepa panicea* L.; *Lasioderma serricorne* F. (Anobiidae); and *Dermestes vulpinus* F. (Dermestidae), no virgin females laid any fertile eggs, and only those of *Tribolium* and *tenebrio* laid any eggs at all.

The present study also shows that in the Scottish race of *Hylecoetus*, females produce smaller eggs (0.88-1.6 mm.) than those produced by females in the Austrian (1.5-2.1 mm.) race of *Hylecoetus*. However, the fewer eggs produced by *Hylecoetus* in Scotland are expected to be larger than those

produced by *Hylecoetus* in Austria. Crowson (1981) noted that given a fixed number of larval instars and a fixed 'Dyar's Law' Constant, one would expect the sizes of the eggs to be proportional to that of the adult beetles interspecifically; intraspecifically, egg size seems to be fairly constant, individual size being reflected more in egg numbers. The larger the number of instars, and the larger the Dyar's Law Constant, the relatively smaller the eggs should be. He added that in Chrysomelidae small eggs tended to be associated with large numbers produced by females, and with increased numbers of ovarioles. The relative selective advantages of many small eggs, with a concomitant lengthening of the larval development, or fewer large ones with faster larval development, doubtless vary with changes in the biotic environment.

At the end of embryonic development, it is necessary for the egg shell to be ruptured in the process of hatching. In many Coleoptera, this function is performed by the mandibles, but there are many others which use specialised 'egg-bursters' (oviruptors), in the form of sclerotized teeth or sharp edges, situated on various parts of the body in different groups. In almost all the Adephaga, as far as is known, there is a pair of egg-bursters on the frons of the first instar (van Emden, 1946). Egg-bursters are found in some Polyphaga e.g. Hydrophiloidea (Crowson, 1981). Egg-bursters have not been previously reported in the first instar larvae of *Hylecoetus*. However, Kurir (1972) and Egger (1974) figured the first instar larva of *Hylecoetus*, and indicated that the last abdominal segment has a shield with two sclerotized, hooked spines. The function of the spines has not been previously described. However, their possible role as 'egg-bursters' should be considered. Kurir (1972) observed first stage *Hylecoetus* larvae feeding on their chorions, a characteristic which possibly indicates that first stage larvae use mandibles to rupture their chorions.

A range of measurements of body length showed that the larva measures 2.38 to 25.26 mm.; with headcapsule width of 0.48 to 2.88 mm. (n=372). Crowson (1981) reported that abnormal patterns of development, such as hypermetamorphosis, paedomorphosis or neoteny, and paedogenesis, occur in certain groups of Coleoptera. The term hypermetamorphosis is usually used to indicate the occurrence of two or more sharply different larval forms; in its commonest form the first instar larva differs widely from subsequent ones, usually in being adapted for high mobility, whereas later instars are very sedentary. In *H. dermestoides* the first instar larva has 5 ocelli, and the ninth abdominal segment is represented by a horny shield, whereas later instars have 'tails', and eyes are wanting. Crowson (1981) reported that hypermetamorphosis occurs in some Lymexylids.

During the larval development the gallery is not widened all the way to the exterior; the larva pushes the bore-meal through the little hole in the outer layer of the bark. Galleries exposed by removing the bark are immediately plugged by the larvae, but a narrow passage is left through which the 'tail' may be seen projecting when the bore-meal is being expelled. Adult *Hylecoetus* enlarge the hole in the bark (Fergusson, 1920). However, Thomsen (1950) observed that the larva enlarges the diameter of the tunnel (1.5-2.5 mm.), while the external orifice retains the original diameter (0.3-0.4 mm.). The present study shows that winter is passed as a second, third or fourth larva instar. However, most of the larvae which are due to pupate during the next spring become last instars before the onset of winter. Fergusson (1920) and Thomsen (1950) reported that winter is passed in the larval condition, but they did not indicate which instars overwinter. Overwintering or diapause is a more or less prolonged suspension of feeding and locomotory activities, with reductions in the oxygen consumption and the basic metabolic rate, together with an arrest or regression in the development of reproduction etc. (Crowson, 1981). In

spring the larva widens the external end of the tunnel and the hole in the bark (2.3–3.8 mm. diameter), and a plug of frass is made to close the outer end of the pupal chamber (Thomsen, 1950). This study shows that there may be 5 instars for female and 5 for male *Hylecoetus* larvae. Under controlled conditions (20°C.), the average period between the fourth and the fifth instars was 25.6±6.6 days. However, if in nature larval stadia were of the same or similar duration then a minimum of 73 days (365/5 stadia) between larval instars would be expected for a 1 year life-cycle. Therefore, 26 days for a larval stadium seems to indicate that a considerable reduction in the duration of the larval stadium and indeed the duration of the life cycle. However, whether the observed 26 days between the 4th and 5th instars represents the actual situation in the field is not known.

As a result of the study of 28 species of Lepidoptera, Dyar (1890) came to the conclusion that the rate of growth in successive instars, as measured by the increasing size of the headcapsule, was in regular geometric progression. Fisher's (1924) work on *Tortrix pronubana* Hb. confirmed this conclusion. Taylor (1931) working with a Tenthredinid larva, *Phyllotoma nemorata* (Fallen), concluded that, Dyar's rule cannot be employed without reservation, and direct observation is the most satisfactory method of determining the number of instars in a given larva.

Dyar's rule was used successfully in a study of the larvae of *Dendroctonus pseudotsugae* by Bedard (1933). He found that headcapsule measurements fell into distinct groups so that the number of instars was clearly indicated. Gaines and Campbell (1935) discussed two generalizations which may be traced back to Dyar (1890) as follows:

1. The sclerotized parts of insects do not change in area during a stadium; the increase in areas of these parts during the larval development only occurring at ecdysis.

2. The discontinuous or step wise increase in the dimensions of the sclerotized parts of insects during larval development usually takes the form of a geometric progression (Dyar's rule).

These authors reported that from the first generalization it follows that any instar of a species can be characterized by a dimension of a sclerotized part of the body of an individual of that instar, provided the range of variation of the dimension among individuals of the instar does not overlap that of the next preceding or succeeding instar. Zethner-Møller and Rudinsky (1967) plotted measurements of 960 larvae of *Hylastes nigrinus* (Coleoptera:Scolytidae) and found 5 modes which represented 5 instars. These authors then assumed and managed to prove that the headcapsule width growth displayed a geometrical progression (Dyar's rule), and made a theoretical calculation of the width of each instar, founded on the known width of the first and last instar. The differences between the values actually found and those theoretically calculated were 7% or less.

The number of instars characteristic of a given species may be determined in many ways, including direct observation of larvae reared through the entire larval stage and through plots of frequency distributions of headcapsule widths taken from larvae representative of the entire larval stage of the species. The latter procedure will provide a multimodal curve with each peak being representative of headcapsules found within one instar. The total number of peaks exhibited for the species in the collection of larvae examined will represent the number of instars for that species. However, it has been noted, based on several studies, that the frequency distribution does not always show a clear separation of each instar. It has often resulted in: (1) *uncertain estimation* [*Monochamas alternatus* Hope (Coleoptera:Cerambycidae): 4 or 5]; (2) *an equal estimation* [*Cryptorrhynchus lapathi* L., 4, 5 and 6]; and *impossibility of estimation* [*Hylobius abietus* L.]. The number of moulted

headcapsules of *Pissodes abietus* Roelofs (Coloeptera:Curculionidae) is 4, but the frequency distribution of the headcapsule widths and lengths of the weevil larvae do not always show 4 clear separations. Conclusions drawn from earlier studies show that it is necessary for more reliable determination of larval instars to count the moulted headcapsules in the larval gallery and in the pupal cell (Kishi, 1971; Schmidt, Campbell and Trotter, 1977).

Despite the limitations of Dyar's rule in some groups, such multimodal curves, and the resultant classification of the number of instars characteristic of a species have appeared in the literature since 1928: *Laspeyresia* = *Cydia* Busck. (Lepidoptera:Tortricidae) (Peterson and Haeussler, 1928); and are in current use: *Hylastes nigrinus* (Zethner-Møller and Rudinsky, 1967); *Xylosandrus compactus* (Eichhoff) (Ngoan *et al* ., 1976); *X. germanus* (Eichoff) (Hara and Beardsley, Jr., 1979) etc.

The present study shows that Dyar's rule cannot be easily applied to *H. dermestoides* due to the sex differentiation by size. However, direct observation coupled with separation of females and males during the pupal stadium would render Dyar's rule useful in studying larval instars in *Hylecoetus*. Plots of frequency distributions of morphological characters of *Hylecoetus* larvae were unsuccesfully used to determine the number of larval instars. It is interesting to note that Dyar's rule is applicable to Coleoptera in which the sexes are not differentiated by size, in contrast to sex differentiation observed in *Hylecoetus* eggs, larvae, pupae and adults.

Pupation takes place in that part of the gallery nearest the bark, or entirely in the bark (Fergusson, 1920; Thomsen, 1950). The pupal stage only lasts for about 7 days, but the callow adult remains for a few days in the tunnel (Thomsen *et al* ., 1949; Thomsen, 1950). The present study confirms that the pupal period is about 7 days. A total of 10 pupae (8 female and 2 male)

with a pupal period of 6–8 (mean=7.2) days at 20°C. A statistical comparison of the pupal period in female and male *Hylecoetus* was not carried out due to the small number of male pupae obtained. Gadd (1947) reported that the pupal period of the ambrosia beetle *Xyleborus fornicatus* Eichhoff (Scolytidae) was influenced by temperature: at 28°C. the pupal period of the tea beetles varied from 4 to 6 days, a mean of 5.2 days, but at room temperature the period extended from 9 to 11 days (mean=9.9 days).

It appears from Thomsen's (1950) description that adult *Hylecoetus* removes the plug of frass, or gnaws the bark to enlarge the exit hole (Fergusson, 1920). The present study shows that normally the external orifice retains the original diameter and it is only enlarged by adult *Hylecoetus*, thus confirming Fergusson's (1920) earlier observation. The pupa remains near the bark during warm spells, but retreats to the end of the tunnel during periods of inclement weather, or when galleries are exposed by removing the bark (Section 2.1.4.4).

The precise duration of the different stages in the life cycle of *H. dermestoides* have not been determined because of the difficulty of breeding them under conditions allowing their direct observation. However, the data obtained in the present study show that part of the *Hylecoetus* natural population completes the life cycle in 1 year, while the rest do so in 2 years. *Hylecoetus* bred in larch and spruce stumps had a similar pattern of development. There is also circumstantial evidence that *Hylecoetus* may attack hosts already infested with *Hylecoetus* broods from earlier generations. The middle instars, 2nd, 3rd and 4th, mark the longest period of development. Under controlled conditions, the duration of the life cycle varied from 9 to 12 months or longer. The duration of the larval period seemed to be influenced by temperature (20°C.). Gadd (1947) found that in *Xyleborus fornicatus*, the larval period is affected by temperature. Earlier workers hold that the life cycle of

Hylecoetus lasts one year. Fergusson (1920) reported that in Scotland, the generation is normally an annual one, whereas Thomsen (1950) observed that *H. dermestoides* needs at least two and possibly three years to complete its development.

A one-year life cycle is common in Coleoptera of nearly all groups, and is probably an ancestral condition in the order, at least outside the tropical rain forest belt. It has the advantage of permitting the close adaptation of particular stages in the life-cycle to the climatic and other conditions of particular seasons; clearly a long-term advantage to any species living in a markedly seasonal climate. As soon as a generation comes to more, or less, than a year, this advantage is lost. Where the life cycle takes more than one year we find that two or more winters (or dry seasons) are passed in the larval stage, while pupae, adults, eggs and first instar larvae occur at fixed seasons within a single year, so that the advantages of seasonal adaptations are retained to a considerable extent. The commonest departure from the one-year norm in beetle life cycles is to a life cycle of two or more years. This is common in wood-boring species, also in soil-living Scarabaeidae, Elateridae, and Cantharidae etc. In general, species showing it tend to be medium size or large size and to have short-lived adults (Crowson, 1981). In *Hylecoetus*, part of the population completes the life-cycle in one year, one to two winters are passed as 2nd, 3rd or 4th larval instars, whereas pupae; adults, eggs, first instar larvae and last instar larvae occur at fixed seasons within a year. If mortality of part of the population occurred, e.g., through attack by specialised predators or parasites the chances are that the remainder of the population will ensure the pertuation of the species. This means that the completion of the life-cycle in one or more years ensures that the advantages of seasonal adaptations are not lost.

At Dunkeld (in both Atholl and Craigvinean forests) the activity of

H. dermestoides starts in spring when the sun heats the bark of stumps, exposed roots and standing dead or dying trees in which callow adult *Hylecoetus* appear. Emergence starts when air temperature has reached about 15°C. Thomsen *et al* . (1949) reported that under laboratory conditions *Hylecoetus* initiated flight after temperature had reached 10°C. Emergence may also be related to light and aspect of exit holes. More *Hylecoetus* emerged from cages facing the N-E aspect than from those cages facing the S-W aspect. Usually the N-E has low morning sun and the S-W has low afternoon sun. Therefore, the N-E aspect would be warmer in the afternoon, and these warm afternoon periods coincide with the main *Hylecoetus* activity. The comparison of daily emergence with total daily catch shows that flight is significantly correlated with emergence. This result shows that *Hylecoetus* were in flight the day they emerged. Both emergence and flight were not recorded on days with unfavourable weather conditions. However, a few *Hylecoetus* were collected from rearing cages in the field on days without flight. This was probably due to the fact that pieces of wood in the box were exposed to warmer conditions than those around stumps in the field. Therefore, *Hylecoetus* in the caged pieces of wood would emerge, but probably be unable to fly, whereas *Hylecoetus* in stumps would remain in their galleries.

Fergusson (1920) reported that in Scotland *Hylecoetus* emerge between May and July; Eckstein (1929a) reported that in West Germany *Hylecoetus* flight occurred in April-June; in Denmark *Hylecoetus* appear during the last days of April or the beginning of May (Thomsen, 1950), whereas in Austria flight occurred from 1st April to 18 May, with peak flight occurring between 11 and 13 April (Kurir, 1972). In 1984-1985 emergence of *Hylecoetus* at Dunkeld occurred from April or May to June. These observations agree in part with those given by Ferguson (1920). The difference between the flight period for *Hylecoetus* in Scotland and flight periods of *Hylecoetus* on the Continent may

be due to geographical variation. Climatic factors influence the activity of bark beetles (Rudinsky, 1962). Bakke (1968) reported that in Norway climatic conditions vary considerably: for example, spring starts several weeks earlier in the south than in the north. He studied the flight of bark beetles at different localities in Norway between 58° and 69°N (page 271). He also found that temperature was suitable for flight in early spring in the south and most *Tomicus* activity occurred earlier whereas temperature was suitable for flight later in the north. When temperature was suitable in a period in early spring most of the *T. piniperda* population started flying (at 58°). After the main flight period the number of flying *T. piniperda* was rather small even if the temperature was suitable. In addition, the highest maximum temperature within 5 days before the first trapping was lower, the maximum temperature on the day with the highest catch was higher at lower latitudes. By inference results obtained in this study show that emergence and flight of *Hyleoetus* seems to be earlier at lower latitudes (Austria, Denmark and W. Germany) and later at higher latitudes (Scotland). In Finland flight data obtained by Löyttyniemi and Uusvaara (1977) indicate that *Hylecoetus flabellicornis* flies earlier in the south and later in the north.

At Dunkeld in 1984–85, seasonal temperature, hours of sunshine and relative humidity were the most important weather parameters influencing the numbers of *Hylecoetus* caught in flight. High air temperature ($\geqq$15°C.) and hours of sunshine were favourable for flight, whereas high relative humidities were negatively correlated with numbers of flying *Hylecoetus*. Wind speed and rainfall may be important weather factors adversely affecting the numbers of *Hylecoetus* in flight. The interactions of weather parameters influenced the numbers of *Hylecoetus* in flight to different degrees. For instance while temperature and sunshine (by inference light intensity) may be favourable for flight, few or no *Hylecoetus* would fly if the relative wind speed was high.

Although hours of sunshine were significantly correlated with the flight of *Hylecoetus*, some days with high humidities and long periods of sunshine had litle or no *Hylecoetus* flight because the temperature was not favourable for flight i.e. it was below 15°C. It follows that days with high humidities and strong winds are expected to have the least numbers of *Hylecoetus* in flight, because high humidities and strong winds were negatively correlated with air temperature and hours of sunshine respectively. Days with high air temperatures and long periods of sunshine were the most favourable for flight because on such days both the %R.H. and wind speed were low. However, when considered as pair, air temperature and relative wind speed are not a significant correlate with *Hylecoetus* flight. It appears, therefore, that the interaction of temperature and hours of sunshine, was seasonally the most important positive correlate with, or trigger for, *Hylecoetus* flight.

The numbers of *Hylecoetus* caught in flight in a day were positively and significantly correlated with: (1) the mean daily temperature and the maximum air temperature. Other aspects of the temperature factor do influence the flight activity of *Hylecoetus* during the course of a flight day. The number of hours the temperature exceeded the threshold (15°C.), and the temperature range above 15°C. given in degrees were important correlates with *Hylecoetus* flight. These results are similar to those reported by Bakke (1968) for Scolytid bark beetles. He demonstrated that the flight of bark beetles depends on air temperature. At the lower threshold necessary for flight the activity can start, and above this temperature flying activity will increase up to a certain temperature level if there are beetles in the forest ready for flight. In early spring a too low temperature is a limiting factor for flight activity. For some days the temperature may rise just above the threshold temperature for flight for only a short time, then the flight, and hence the capture of beetles, will be rather small. However, if the temperature just exceeds this threshold

temperature for a long period of the day, the beetle catch will be improved. On the other hand, flight intensity and the number of beetles taken in the trap may increase if the temperature rises to the most suitable temperature for flight for only a few hours. Thus flight intensity in a day and daily capture of beetles depends on at least two temperature factors: (1) the period of time with air temperature above the threshold temperature for the flight; (2) and the level of the air temperature during this period. In the present study the correlation between catch of *Hylecoetus* and maximum air temperature was clear on most days, except May, 20 1984 when the catch of *Hylecoetus* was lower than on 28 May, 1984 in spite of both dates having more or less the same maximum air temperature. On 29 May the temperature was above 15°C. for only 3 hours, whereas 28 May had 6 hours above that threshold. The present study shows that emphasis should be on the level of air temperature above the threshold necessary for flight, and the duration of time the temperature is above the threshold for flight, and these temperature factors are both more important correlates with *Hylecoetus* flight than maximum temperature. McLean and Borden (1979) noted that the favourable temperature for flight of *Gnathotrichus sulcatus* is between 15° and 21°C. The former workers demonstrated that the number of *G. sulcatus* captured and/or suppressed using racemic sulcatol (pheromone)-baited traps, were significantly correlated with the number of flight hours with temperature above 15°C. and 21°C., flight hours 15°–21°C. (with the difference between the two giving the number of favourable hours), and the number of degree hours above 15°C. (obtained by calculating the area under a thermograph trace above 15°C.).

The flight pattern of *Hylecoetus* varies at different times of the day. *Hylecoetus* is limited in its flight activity by low temperatures in spring. *Hylecoetus* needs about $\geqq$15°C. in order to fly. On most flight days the temperature was above this threshold for flight only for a few hours in the

morning and in the evening. In the morning frequently low temperature, high wind speed and high relative humidity prevented flight activity. In the evening the temperature was usually above 15°C. for a few hours, especially during the latter half of the flight season. It seems that high wind speed, high humidities and low light intensities prevented the evening flight of *Hylecoetus*. Thus an interaction of temperature $\geqq$15°C. and high light intensities seems to be the main factor which determines the diurnal flight of *H. dermestoides*. Rudinsky and Daterman (1964) reported that *Trypodendron lineatum* had two distinct peaks in flight during a warm day, one in the afternoon or at noon, and the other in the late afternoon. They found that *T. lineatum* was prevented from flight activity when the temperature was above 30°C.(86°F.). However, in the present study there was no trend in the data of flight activity which indicated reduced activity in the middle of the day because of high temperature. Air temperature never exceeded 26°C. in 1984-85, and there is no reason to believe that normal high temperatures under field conditions will prevent flight of *Hylecoetus* in the middle of the day.

Studies on host location were primarily concerned with the mechanism by which *Hylecoetus* locates its preferred breeding material, and secondarily, only by way of field observation, to obtain knowledge on where and how *Hylecoetus* finds partners for mating. Answers to these questions would not only contribute to our knowledge of olfactory responses in Lymexylids, but would also contribute to the control or prevention of damage to timber. Observations on flight behaviour showed that *Hylecoetus* generally flew at 2-3 m. above the ground and made random flights. However, when *Hylecoetus* was in proximity to suitable hosts, the flight became firmly directed and there was a loss in height before eventual landing on the hosts. The flight behaviour of *Hylecoetus* prior to landing on the hosts is similar to that described by Rudinsky and Daterman (1964) for *Trypodendron lineatum* as klino-kinetic along

the concentration of the attractant. Both female and male *Hylecoetus* landed on the same hosts, whereupon after mating, females laid eggs. It was also observed that both female and male *Hylecoetus* may land on preferred hosts at the same time.

Odours emanating from dead or dying trees or stumps etc. are known to be essential in guiding ambrosia beetles to their hosts (Francia and Graham, 1967; Graham, 1968). Since *Hylecoetus* attacks felled or dead trees and stumps only after the material has aged, it seems most likely that chemicals evolved during the process of log decomposition are responsible for *Hylecoetus* attraction. Graham (1968) reported that anaerobic fermentation accounts for production of chemicals attractive to ambrosia beetles. Decomposing conifer logs, stumps etc. produce chemicals which are attractive to ambrosia beetles. The attractancy in the host material is induced by the products of fermentation. Induced anaerobiosis in wood and bark of conifers produces primary chemical attractancy (Moeck, 1970). Isolates (vapour trapping) from anaerobically treated wood and bark, and similar material under attack in the field have been identified (gas chromatography) as being composed of principally monoterpenes (natural products) and fermentation products viz. alcohols (ethanol, methanol) and acetaldehyde. Bioassays and field trapping of beetles show that ethanol is the primary attractant for the ambrosia beetles *Trypodendron lineatum* and *Gnathotrichus sulcatus*. Ethanol alone, or synergizes with the host monoterpene α-pinene to attract beetles to hosts. Secondary attraction in Scolytids is by the aggregation pheromones, lineatin and sulcatol respectively. A brief survey of tree species carried out by Moeck (1970) showed that all were capable of producing ethanol under the anaerobic conditions. The species tested were the following:Douglas-fir, *Pseudotsugae menziesii*; Western hemlock, *Tsuga heterophylla* (Rafinesque) Sargent; amabilis fir, *Abies amabilis* (Doug.) Forbes; alpine fir, *Abies lasiocarpa* (Hook.) Nutt.; western red cedar,

Thuja plicata Donn.; Shore pine, *Pinus contorta* Doul.; Pacific yew, *Taxus breviflora* Nutt.; trembling aspen, *Populus tremuloides* Michx.; and a chestnut, *Castanea* spp. This list shows that probably, many tree species are capable of producing ethanol under the proper circumstances. Samples of Douglas-fir, western hemlock, and amabilis fir logs under active ambrosia beetle attack in the field also contained ethanol, strengthening the view that ethanol is the primary attractant under natural conditions. Cade *et al* . (1970) isolated three compounds from anaerobically treated western hemlock logs: one of the compounds was ethanol. They carried out field tests of the response of *Gnathotrichus sulcatus* to ethanol, and produced results which indicate ethanol to be a primary attractant for *G. sulcatus*. Dethier (1947) reported that insects attracted by the products of fermentation are not rigidly specific in their feeding habits. The available compounds, however, are limited in number as compared with those in essential oils. Thus there is a similarity about the odours of fermenting plant products as contrasted with numerous characteristic odours of living plants that does not make for specificity among insects on such substances. This is one of the reasons why multitudes of wood-boring insects are attracted to the same baits. *H. dermestoides* and *T. lineatum* have overlapping ranges, both attack and infest stumps, logs, standing dead trees in the spring and summer. Results in this study show that the temperature threshold for flight of *Hylecoetus* is about 15°C. In *Trypodendron lineatum*, spring flight may be expected when maximum daily temperature exceeds 15.5°C., with heavy flight beginning when the air temperature climbs to 21°C. (Chapman and Nijholt, 1980). *Hylecoetus* and *Trypodendron* usually share the same habitat and they are active at the same time of the year, i.e. their flight and response periodicities overlap, and they frequently breed in the same piece of host material (Bletchly and White, 1962). This close relationship between the two ambrosia beetles made it necessary to assume that they may have similarities in their host location process. Although there is no record

indicating that ethanol is a fermentation product of *Larix decidua* under natural conditions, the list of species which produced ethanol under anaerobic conditions (Moeck, 1970), the wide host range for *Hylecoetus* (e.g., Fergusson, 1920; Kurir, 1972 etc.), and the response to ethanol by *Hylecoetus* recorded in this study and by Klimetzek *et al* . (1986), indicate that *Larix decidua*, and indeed other hosts for *Hylecoetus* at Dunkeld, e.g., spruces, may produce ethanol under field conditions, and ethanol with or without host tree monoterpenes, may induce primary attraction for *H. dermestoides*. *Hylecoetus* attacks have been recorded in both conifer and broad leaf genera, and, therefore, this seems to indicate that the physiological condition of the host is of greater importance than its taxonomic affinities in relation to susceptibility to attack by *H. dermestoides*.

Fermentation products such as alcohols, aldehydes etc. may be the by-products of the action of micoorganisms (bacterial, yeasts and moulds) on plant and plant products. Such organisms have enzymes which perform fermentation on these substances (Dethier, 1947). However, there is no record of microorganisms acting on wood to produce attractants for ambrosia beetles.

The field tests utilizing logs, stumps and standing dead larches and semiochemical attractants made it possible to obtain evidence which seems to explain the mode of attraction of *Hylecoetus* to suitable hosts. The present study confirms results obtained by Bletchly and White (1962) who reported the attractiveness of autumn felled logs to *Hylecoetus*. However, the importance of felling date in relation to subsequent attack was not strongly established. Only small numbers of *Hylecoetus* were attracted to larch logs felled in the autumn (November, 1983) and early spring (March, 1984). Larch logs felled in April, 1984 were not attractive to *Hylecoetus* during the 1984 flight season. These results although based on small counts of beetles, also show that autumn felled logs are attractive to *Hylecoetus*, six months after felling, whereas in spring, logs

felled one month before the beginning of flight, may be attractive to *Hylecoetus*. However, logs felled during the flight season are not attractive. This means that a period of one to five months may be required for the ageing of logs before *Hylecoetus* begin flying in April or May. Logs felled during the flight season of *Hylecoetus* become heavily infested by most Scolytid bark beetles. Other insect species attracted to ethanol and larch logs were not identified.

After 13 May, 1984 none of the larch log-baited traps, and larch-stump-baited cross vane traps remained attractive to *Hylecoetus*. The decreased attraction of *Hylecoetus* to logs and stumps seemed to be due to the response of *Hylecoetus* to the presumably more attractive standing dead larches and the ethanol-baited window trap. Considering only the standing dead larches, it can be seen that after 23 May, 1984, trees 1, 3, 4, 6 and 7 were not attractive to *Hylecoetus*, whereas trees 2 and 12 remained attractive up to the end of the flight season. This result indicates that host material such as standing dead larches could remain attractive to *Hylecoetus* throughout the flight season, provided that there is no counter attraction from other sources. In the same area, *Hylecoetus* attacks hosts of varying degrees of attractiveness. Greater numbers of *Hylecoetus* will respond to the more attractive hosts, whereas weakily attractive hosts will receive very few or no *Hylecoetus* at all. The data obtained in this study do not show whether the noticeably reduced attractiveness of certain host material was due to an increase in the number of attacks on such host material or not. In *Trypodendron lineatum* attacks tend to reduce the attractiveness of logs to later *Trypodendron* flights. If logs are well attacked by early flight they are not attractive to parent *Trypodendron* leaving their galleries for re-attack (Dyer and Chapman, 1965). However, in *Hylecoetus* attacks do not seem to affect the attractiveness of host material such as stumps to later *Hylecoetus* flights: it actually seemed possible that the same

stumps may remain attractive to *Hylecoetus* for at least two years. Thereafter, the reduced attractiveness in the stumps may be more due to the ageing factor of the stumps than to the presence of brood material from earlier generations. Aged stumps may, in addition, not be suitable for ambrosia fungal growth. The ambrosia beetle *Gnathotrichus sulcatus* has a preference for stumps over logs (McLean and Borden, 1977); *Tyrpodendron lineatum* has a preference for logs over chunks or tops (Dyer, 1963); whereas the present study does not show whether or not *Hylecoetus* have a preference for stumps, standing dead trees or logs.

The present study shows that *Hylecoetus* responds to ethanol-baited traps and that the response may be dosage-dependent. The results confirm that *Hylecoetus* may have a dosage-dependent response to ethanol as reported by Klimetzek *et al* . (1986). They reported that the response of *Hylecoetus* increased almost linearly with an increase in ethanol concentration. Similar responses to ethanol in *Trypodendron lineatum, Xyleborus dispar, X. saxeseni, Xylosandrus germanus* and *Hylurgops palliatus* were recorded. They, in addition, reported that in *Tomicus piniperda* the response to host monoterpenes decreased with an increasing ethanol concentration, and the addition of ethanol to the pheromone of *Ips typographus* led to reduction of the response at all concentrations. In *Leperisinus varius* F., a low concentration of ethanol resulted in a highly synergistic effect with the male pheromone *exo*-brevicomin, resulting in a high number of female beetles being trapped. Higher concentrations of ethanol strongly reduced this response. They concluded that high ethanol concentrations enhance the response of non-aggressive (secondary) Scolytids and Xyleborins to aggregate on host trees, but interfere with the pheromone response among aggressive species.

The present study shows that ethanol with or without camphene was attractive to *H. dermestoides*. It seems that ethanol with camphene may be the

primary attractant. Incidental results in the present study indicated that *Hylecoetus* responded to glass jars which had contained ethanol and were hidden in forest litter. Female *Hylecoetus* responding to the hidden ethanol-contaminated jars did not fly away after landing in the location of the jars. The attraction of *Hylecoetus* to the immediate area of the hidden ethanol-contaminated jars, indicated that *Hylecoetus* are capable of orienting to sources of chemical stimuli (odours) in the absence of normal visual cues. Ethanol vapours from the hidden jars seemed to arrest female *Hylecoetus*. McLean and Borden (1977) hypothesized that the major role of ethanol is that of an arrestant/stimulant. It acts as a tunneling stimulant for *Xyleborus ferrugineus* (Norris and Baker, 1969b). There was very slight response of *Hylecoetus* to a chloroformic solution of camphene.

Trypodendron lineatum, Gnathotrichus sulcatus, and *G. retusus* use a mixture of ethanol and alpha-pinene in the primary attraction (Borden, 1982). He noted that camphene is a kairomone in *Dendroctonus pseudotsugae* where α-pinene, camphene and ethanol were all components of attractive mixtures. Rudinsky (1966) demonstrated the attractiveness of camphene, in decreasing order, to species of bark and ambrosia beetles associated with Douglas-fir: *Dendroctonus pseudotsugae; Gnathotrichus sulcatus; Hylastes nigrinus; Pseudohylesinus nebulosus* (LeConte); *Trypodendron lineatum; Hylastes ruber* Swaine; and *Dryocoetes autographus* Swaine. The attractiveness of camphene (including α-pinene, β-pinene, Douglas-fir oleoresin and bark) to *Hylastes nigrinus* was reported by Zethner-Møller and Rudinsky (1967). They reported that *Hylastes* species are found breeding in the roots and bases of dead or dying trees or stumps and roots of cut trees and sometimes attack windblown trees, logs and slash in contact with the soil. Several other workers have reported the attractiveness of camphene to Coleoptera: in *Dendroctonus pseudotsugae; Gnathotrichus retusus; Trypodendron lineatum; Xyleborus dispar*

and *X. saxeseni* (Rudinsky *et al* . 1972); *Dendroctonus brevicomis* (Pitman and Vité, 1971), etc. The present study shows that *H. dermestoides* is a non-aggressive species which breeds in diseased or weakened trees, as well as in stumps and logs where anaerobic fermentation leads to the formation of ethanol (Graham, 1968; Moeck, 1970; Cade *et al* ., 1970). Host location and colonization for reproduction occurs in a time and space pattern that seems neither congruent with a pheromone-governed mass aggregation as usually obtained in bark beetles colonizing host trees in response to insect-borne attractants (Borden and Stokkink, 1973), nor in accord with the chain of events in the host location process observed in insects visiting unimpaired host plants. Instead, flights in spring and summer lead to instant and silmutaneous colonization of suitable breeding material, which may or may not be related to previous successful *Hylecoetus* attacks. This silmutaneous arrival of both sexes on prefered host material facilitated mate-finding and oviposition. The obvious attractiveness of uninfested hosts to *Hylecoetus* in flight suggests that host recognition may be due to primary host odours, camphene and ethanol. The attraction of *H. dermestoides* to ethanol with or without camphene seems to explain why *Hylecoetus* attacks have not been recorded in healthy trees. Ethanol is present in dead or weakened pines but absent altogether or present in lesser amounts in healthy trees (Cade *et al* ., 1970; Crawford and Baines, 1977).

The present study does not show evidence of the existence of a pheromonal component in *Hylecoetus* mate-location as suggested by Francke *et al* . (1984). However, work carried out in Germany shows that *H. dermestoides* produces a sex-specific cyclic enolether, 2,5-dimethyl-2-isopropanol-2,3-dihydrofuran, which may be a component of the female sex pheromone (Francke *et al* ., 1984). If a pheromonal component is actually used in *Hylecoetus* secondary attraction, then it is principally employed

in the ultimate location of females by males. Male *Hylecoetus* with their maxillary palpi expanded, pursued females for mating. The results show circumstantially, that the enlarged organs on the male *Hylecoetus* maxillary palpi may play an important role in both primary and secondary attraction processes.

In 1984 8 *Hylecoetus* (4 female, 4 male) were collected from larch log-baited window barrier traps, whereas only one male was found among the 19 *Hylecoetus* attracted to larch stump-baited cross vane traps; 253 (215 female, 38 male) *Hylecoetus* were attracted to standing dead larches; 44 (18 female, 26 male) *Hylecoetus* were attracted to an ethanol-baited window barrier trap. In 1985, the different semiochemicals used attracted a total of 21 *Hylecoetus*, of which 19 were females and 2 males. The larger number of females collected from dead trap trees was due to the fact that most males remained on the attractive hosts for a short while before flying off, whereas females usually did not fly away again. More male than female *Hylecoetus* responded to the 1984 ethanol-baited flight trap. This slightly stronger response of male *Hylecoetus* is difficult to explain in the light of previous studies. MacConnell *et al* . (1977) caught a total of 77 (sex ratio ♀1:0.4♂) *Trypodendron lineatum* in traps baited with ethanol. These results show that the response to ethanol is stronger in *G. sulcatus* in the pheromone-producing males, and stronger in the pheromone-producing female *T. lineatum*. Borden, Lindgren and Chong (1980) reported that ethanol and α-pinene together acted as synergists for male *Gnathotrichus sulcatus* response to (±)-sulcatol. It is not known whether ethanol and camphene would synergize male *Hylecoetus* response to the female attractant.

From its habits and habitat the *Hylecoetus* larva would be assumed to be a genuine wood-borer, ingesting solid wood and digesting at least some part of it. However, Chapman (1978) in collating several previous studies noted

that all Lymexylids studied possess ectosymbionts. The present investigation corroborates that of earlier investigations of symbiosis of ambrosia beetles and fungi. Studies carried out on the European Continent have reported that *Hylecoetus* larvae feed on an ambrosia fungus (Eckstein, 1929a; Batra, 1963a; Batra and Francke-Grosmann, 1961, 1964; Kurir, 1972). The ambrosia fungus for *H. dermestoides* was described as *Ascoidea hylecoeti* (Batra and Francke-Grosmann, 1961). *Ascoidea hylecoeti* has been isolated from various sources: larval and pupal tunnels; egg-shells (Kurir, 1972); larvae; and adults (Batra, 1963a). Propagules of *A. hylecoeti* are found in mycangia of female *Hylecoetus*. Ambrosia fungi spores are found in the frass of ambrosia beetles of the genus *Xyleborus* (Peplinski and Merill, 1974). The results of the present study show that: (1) the Scottish race of *Hylecoetus* is associated with microorganisms, with *Ceratocystis piceae* being the principal associate. *C. piceae* was isolated from all the possible sources of ambrosia fungal inocula listed above. Dissections of mycangia of female *Hylecoetus* were not carried out in this study. However, *C. piceae* was isolated from live adult *Hylecoetus*. *A. hylecoeti* was not found in all the isolations of fungi from the various inocula used in the this study. The absence of *A. hylecoeti* in wood inhabited by *Hylecoetus* was shown by work carried out by Bakshi (1950). The former worker isolated *Leptographium lundbergii, C. piceae* and *Oedocephalum lineatum* from guts of *Xyloterus lineatum* and *Ceratocystis ambrosia* from *X. domesticus*, and regarded them as ambrosia. Although Bakshi (1950) did not investigate the association of these fungi with *Hylecoetus* any further, the absence of *A. hylecoeti* in wood infested by *Hylecoetus* and *Xyloterus* species, gives speculative evidence that if the Scottish race of *Hylecoetus* has ambrosia fungi, then the fungi should be at least some if not all of those listed by Bakshi (1950). This also means that the ambrosia fungi associated with *Hylecoetus* in Scotland may be different from the ambrosia fungi for the Continental races of *Hylecoetus*. This geographical variation in the ambrosia fungi for different races

of *Hylecoetus* has also been reported for *Xyloterus lineatus*. Wilson (1959) recognized *C. piceae* as the ambrosia fungus for *Corthylus columbianus*. Mathiesen-Käärik (1960) reported that the ambrosia fungus for *X. lineatus* on the Continent is *Monilia ferruginea*, whereas Bakshi (1950) found neither *Ascoidea* nor *Monilia* in the ambrosia beetles. The reason for the failure of the present study to isolate fungi other than *C. piceae* in association with *Hylecoetus* is not clear. However, Haanstad and Norris (1985) reported that the number of microorganisms isolated from ambrosia beetles may be influenced by the method of isolation of fungi employed. The adoption of other methods of isolation of fungi from *Hylecoetus* in future studies may reveal that only *C. piceae*, or *C. piceae* and other microorganisms are associated with the Scottish race of *Hylecoetus*.

In the present study *C. piceae* was isolated from traces of field collected adult *Hylecoetus* on agar, indicating a possible and significant role adult *Hylecoetus* have in the transmission of *C. piceae* or indeed other fungi. Whether *C. piceae* is transmitted by adult *Hylecoetus* by simple adherence on body surfaces, e.g., maxillary palpi, legs, elytra, ovipositors or carried in mycangia was not investigated. However, observations by earlier workers on the Continent showed that the ambrosia fungi of *Hylecoetus* may be transmitted by simple adherence onto body surfaces such as maxillary palpi (Eckstein, 1929a) or carried in mycangia. The present investigation does not show whether or not the spores of *C. piceae* carried by adult *Hylecoetus* are introduced into larval tunnels. However, it seems possible that during mating in *Hylecoetus* there may be an exchange of fungal inocula between females and males through physical contact, and as already suggested by Kurir (1972) the contaminated female *Hylecoetus* will then pass on the fungal spores to the eggs. Thus it seems possible that both female and male *Hylecoetus* are important disseminators of ambrosial fungal inocula.

The present study shows that traces of wood particles, fungal mycelia and spores were found in the guts of *Hylecoetus* larvae. It thus appears almost certain that the *Hylecoetus* larva feeds on a wood-fungal mycelium complex. The study also shows that only part of the bore-meal actually goes through the body. Francke-Grosmann (1967) noted that in old *Hylecoetus* galleries, the ambrosia fungus growth is regressive in the older tunnels, containing full-grown larvae, and many 'weed-fungi' such as *Ceratocystis* and *Leptographium* spp, or *Isaria, Aspergillus*, and *Verticillium* spp (Lyngnes, 1958) appear, and that fragments of *A. hylecoeti*, weed fungi, and particles of wood are constantly found in the *Hylecoetus* larval guts. These results show that the diet of *Hylecoetus* consists of a wood-fungi complex. However, this study and work by Bakshi (1950) show that the *Ceratocystis* spp found in the Scottish race of *Hylecoetus* do not have a 'weed-fungi' status, but are components of associations of multispecies complexes of ectosymbiotic microorganisms with the ambrosia beetles (cf. Haanstad and Norris, 1985). The present study shows that *Hylecoetus* differs from another Lymexylid *Melittomma insulare* which bores into woody material but only fluids and other flocculent matter pass through the gut, indicating that its food consists, if not, entirely, of fluid obtained normally direct from the parenchyma tissues (Brown, 1954).

C. piceae may have a certain value for the nutrition of *Hylecoetus* larvae, since in the present investigation, *Hylecoetus* larvae were throughout their development associated with *C. piceae*. Larvae of *Hylecoetus* could be reared aseptically, but this seemed to reduce the numbers of viable eggs laid by female *Hylecoetus*.

The effect of blue-stain fungi on wood and their possible role in the nutrition of their insect associates has been studied by Mathiesen-Kääarik (1960) who showed by comparative cultivation of the fungi on different synthetic media that the enzymatic properties of blue-stain fungi exceed the

digestion of the components of the cell contents such as sugars, starch and proteins. He observed a depolymerization of pectins and a limited decomposition of cellulose. Farris (1969) reviewed the work on the effect of *Ceratocystis minor* and *C. ips* on the free sugar pool of Ponderosa pine sapwood and noted that the action of cellulases, hemicellulases, and pectolytic enzymes were responsible for decomposition of the cell wall components resulting in an increase of glucose and xylose and in the production of galacturonic acid, and that hemicellulases have been isolated from blue-stained ponderosa pine sapwood. The present study shows that there were no noticeable differences between the numbers of amino acids and sugars found in larch bark and *Hylecoetus* bore-meal. At least 18 amino acids were found in the fresh larch bark, sapwood and *Hylecoetus* bore-meal. This is far more than previously reported by Balogun (1969) who reported the occurrence in larch bark of only ten amino acids (page 41). Arginine, histidine, lysine and methionine were found only in the *Hylecoetus* frass. This result shows that the *Ceratocystis piceae* infested wood had more amino acids than fresh bark and sapwood. This means that the feeding by *Hylecoetus* on the wood-microorganismal complex, has a nutritional advantage, that is the larvae acquire some amino acids which seem to be lacking or are less available in fresh bark and sapwood. This shows the importance of *C. piceae* in *Hylecoetus* development. The presence of *C. piceae* in larval tunnels presents to *Hylecoetus* larvae a more favourable environment, in terms of nitrogen nutrition. The present investigations suggest that the beneficial relationship between *Hylecoetus* and *C. piceae* is the result of qualitative changes in amino acids of infested tissue (i.e. the synthesis of amino acids not normally present in the uninfested tissue). This also means that the influence of *C. piceae* seems to result in an increase in total nitrogen in the infested tissue. This finding does not preclude the possibility that *C. piceae* may influence *Hylecoetus* development through the changes in chemical compounds other than amino

acids. This result seems to agree with the general notion that ambrosia fungi accumulate nitrogenous compounds required for beetle and larvae nutrition. The present study shows that fungal symbiotes of *Hylecoetus* also provide larvae with essential amino acids such as lysine, methionine, arginine and histidine. This result agrees with reports which show that fungal symbiotes also provide *Xyleborus* beetles with essential amino acids (e.g., lysine, methionine, arginine and histidine) required for the initiation of reproductive processes (Norris, 1979b).

Sucrose, glucose, fructose and raffinose were found in larch bark and sapwood, confirming the observations of Balogun (1969) who found the same sugars in larch bark (page 41). These sugars are components of the sugar pool that may be available to *C. piceae* and *Hylecoetus* larvae.

Ceratocystis piceae grew readily on most of the common laboratory media, with linear extension being most rapid on malt extract plus yeast extract (YEME) and potato dextrose agar (PDA). The rapid linear extension on YEME may be due to the high vitamin content. *C. piceae* grows readily on larch bark, sapwood and heartwood extracts. Linear extension is more rapid on bark than on sapwood and heartwood respectively; and sapwood extracts results in better linear extension than does heartwood. The present investigations show that the differences between bark, sapwood and heartwood in their effect on linear extension of *C. piceae* may be due to the differences in the nutritients. More amino acids were found in bark than sapwood. Linear extension of *C. piceae* was more rapid on a medium with a mixture of sugar and amino acids extracts from larch sapwood than on media with only sugars or amino acids respectively. An organic acid supplement did not have any significant effect on the linear extension of *C. piceae*.

The tests of sucrose, glucose, fructose and raffinose showed that

C. piceae grows on these sugars, and this result agrees with that of Mathiesen-Käärik (1960). Aldopentose sugars resulted in better extension than did aldohexose sugars. There were no significant differences between monosaccharides, disaccharides, trisaccharides and polysaccharides. *C. piceae* grew on all the sources of carbon tested, thus confirming the results of Mathiesen-Käärik (1960). However, the present investigation shows that the effect of carbon sources on the linear extension of *C. piceae* in culture was dependent upon two factors: (1) the combination of sugars; and (2) the concentration of each sugar in the medium.

The present study shows that *C. piceae* requires organic nitrogen and is capable of using inorganic nitrogen. Among the amino acids found in larch bark, sapwood and frass, arginine and leucine resulted in the best and poorest extension of *C. piceae* respectively. Cystine is also a good source of organic nitrogen for *C. piceae*. The effect of a nitrogen source on the linear extension of *C. piceae* depends on the concentration used in the medium.

C. piceae grows on all the inorganic nitrogen sources tested, with ammonium tartrate and calcium nitrate resulting in the best extension, whereas sodium nitrate, potassium nitrate and ammonium nitrate resulted in the least extension. These results show that the linear extension of *C. piceae* in culture is better on ammonium than nitrate nitrogen. This result agrees with results obtained by Mathiesen-Käärik (1960) who reported that nitrate was a poor source of nitrogen for primary blueing fungi which included *C. piceae*. Fries (1975) used ammonium tartrate in the basic medium on which *C. piceae* was grown. A low nitrogen content in the medium favoured the rapid extension of *C. piceae*.

The linear extension of *C. piceae* is affected by the temperature of incubation. *C. piceae* grew at 5°-30°C., with the most rapid extension being at

15°–25°C. Fries (1975) incubated *C. piceae* at 25°C. and Gibbs (1980) grew *C. piceae* at 20°C.

The linear extension of *C. piceae* was most rapid on an acidic medium and showed a wide pH-tolerance in the acid region. Fastest radial extension was at pH's 3–4, and pH's lower than 3.4 inhibited growth. However, abundant coremia were produced at pH's 4.0–6.0. These results agree with those of Fries (1975) who grew *C. piceae* on medium with pH 5.2. Puhalla and Bell (1981) noted that *Ceratocystis* spp are best adapted to low pH values in the range 4.0 to 6.0 (Puhalla and Bell, 1981). Generally, nutrients, temperature and pH affected not only radial extension but also the sporulation of *C. piceae*. Similar results have been reported for: (1) 23 blueing fungi including *C. piceae*, by Mathiesen-Käärik (1960); (2) *Ceratocystis ulmi* and *Ceratocystis fagaceraum* by Hubbes and Pomerleau (1969); Harris and Taber (1970) and Puhalla and Bell (1981).

CONCLUSIONS

CONCLUSIONS

1. *H. dermestoides* are sex differentiated by size and this renders the plotting of frequency distributions of measurements of headcapsule widths or the application of Dyar's rule to the measurements unsuitable to use when determining the number of instars of larvae from natural populations.

2. The length of the *H. dermestoides* larva is 2.38 to 25.26 (mean=9.4) mm.; the headcapsule width is 0.48 to 2.88 mm. (mean=1.26mm)(n=372).

3. During larval development the gallery is not widened all the way to the exterior but the larva pushes the bore-meal through the little hole in the outer layer of the bark.

4. Winter is passed as a second, third or fourth instar larva, i.e. middle instars. The middle instars of *Hylecoetus* mark the longest period of development.

5. Pupae, adults, eggs, first stage larvae and last instar larvae occur at fixed seasons within a year in nature.

6. There is possibly as many as 5 instars for female and 5 for male *Hylecoetus* larvae.

7. The pupal period is 6-8 (mean=7) days at 20°C.

8. Part of a *Hylecoetus* generation completes the life cycle in one, while the rest does so in 2-3 years.

9. The duration of the life cycle can be modified by artificial rearing.

10. The use of emergence boxes in the field gives indication of the emergence pattern for *Hylecoetus*.

11. In *Hylecoetus* emergence and flight coincide and occur from the end of April to the end of June, with maximum activity being between mid-May and mid-June.

12. Both emergence and flight occur on days with favourable weather. Emerging *Hylecoetus* have approximately a 1:1 sex ratio. The female portion of the flying *Hylecoetus* population is greatest at the beginning of the flight season, whereas a 1:1 sex ratio of *Hylecoetus* flying in the middle of the season can be expected.

13. All-black and yellowish brown males occur together, with all-black males being more abundant than the yellowish brown males.

14. Female *Hylecoetus* are significantly larger than males, being 10.3 to 16.4 mm. and 8.2 to 12.7 mm. long respectively.

15. Adults are short lived and lay eggs on the day they emerge or the second day after emergence. Copulation and ectosymbiotes are necessary in *Hylecoetus* for large numbers of viable eggs.

16. The weather parameters which influence emergence and flight of *Hylecoetus* also may influence their mating and oviposition.

17. Numerous events in the life cycle of *Hylecoetus* i.e. emergence, flight, pairing, oviposition etc. occur synchronously with periods of favourable weather. The beginning and end of these activities being influenced by seasonal variation.

18. The temperature threshold necessary for flight of the Scottish race of *Hylecoetus* is $\geqq 15°C$.

19. *Hylecoetus* is a day flyer, under favourable temperature ($\geqq 15°C$.) flight occurs from 10.00 to 20.00HRS, activity is greatest between 13.00 and 15.00 HRS, and corresponds with periods of increasing temperature and by inference increasing light intensity, and decreasing relative humidity.

20. There is apparently variability in the sex ratios of flying *Hylecoetus* throughout the course of a flight day or season. In a day, duration of females is 7–10 hours, whereas males are on the wing for 4–7 hours.

21. Temperature factors: daily maximum or mean; the number of hours during which the temprature exceeds the threshold necessary for flight; temperature range above the threshold for flight; number of hours of sunshine (or light intensity) are possible correlates of flight of *Hylecoetus*, whereas relative humidity and relative wind speed adversely affect *Hylecoetus* activity in the field. The temperature threshold for flight provides *Hylecoetus* with the possibility for locating and colonizing new hosts.

22. Seasonally, temperature and hours of sunshine (light intensity) have a stronger influence on emergence and flight of *Hylecoetus* than other weather parameters examined. The temperature of the soil at the 10 cm. depth, minimum air temperature, minimum grass temperature and rainfall did not significantly influence emergence and flight of *Hylecoetus*.

23. There is one flight in a year with a duration of about 10 weeks.

24. There are differences between the Scottish and Continental races of *Hylecoetus* in the flight periods. These differences may be due to geographical variation. Emergence and flight of *Hylecoetus* are earlier at lower latitudes (Austria, Denmark and W. Germany) and later at higher latitudes (Scotland).

25. An apparently pronounced difference exists between female and male *Hylecoetus* in their reactions to light and temperature; most females fly during long periods of sunshine, whereas males fly during the warmest time of the flight season.

26. After flight several female *Hylecoetus* can be found at their new hosts in the evening, where they remain for oviposition. These large numbers of females do not indicate that *Hylecoetus* are predominantly evening flyers.

27. *Hylecoetus* flight is most noticeable near attractive stumps, logs or standing dead trees. Most flight takes place at 2–3 m. above the ground and *Hylecoetus* make random flights, but fly head to wind when approaching suitable hosts.

28. Field observation and objective data on flight and attack behaviour of *Hylecoetus* lead to the conclusion that *Hylecoetus* are guided to attack by odours emanating from hosts. When searching for hosts *Hylecoetus* fly with head to wind until they are in proximity of the source of attraction. After alighting males search for females, whereas females search for oviposition sites. The study also shows that cues other than those used in primary attraction may be employed in mate recognition.

29. Ethanol with or without camphene may be the primary attractant for *Hylecoetus* in the field. The response of *Hylecoetus* to ethanol-baited flight traps may be dosage-dependent. The response to ethanol is stronger than to camphene.

30. Other monoterpenes in European larch: (1) α-pinene; (2) β-pinene; (3) limonene; and (4) myrcene are apparently not attractive to *Hylecoetus*.

31. *C. piceae* infested wood has more amino acids than fresh bark and sapwood. This shows that the feeding by *Hylecoetus* on the wood-*C. piceae* complex may have a nutritional advantage. *Hylecoetus* acquires certain amino acids, e.g., glycine, methionine, arginine and histidine, which are not readily available in fresh wood and bark.

32. *C. piceae* grows readily on most common laboratory media; mixtures of sugars and amino acids found in larch extracts. Sucrose, glucose, and fructose are more important than raffinose for radial growth of *C. piceae*. The influence of sugars and amino acids on the growth of *C. piceae* depends on the combination, and the concentration of each nutrient used in the medium. Arginine, leucine and cystine result in the most extensive growth of *C. piceae* in culture. The extension rate of *C. piceae* in culture is more rapid on ammonium than nitrate nitrogen.

33. Nutrients, temperature of incubation and pH of the medium influence both radial extension and sporulation by *C. piceae*. Temperature between 15°-25°C., and pH's 3-4 result in the most rapid linear extension of *C. piceae*.

34. *Ascoidea hylecoeti* the ambrosia fungus for Continental and United States races of *Hylecoetus* is apparently not an ambrosia fungus for the Scottish race of *Hylecoetus*.

SUMMARY

SUMMARY

The main objectives of the present study have been to elucidate the biology of the Scottish race of *Hylecoetus*, special emphasis being laid on the duration of the life cycle, number and length of larval stadia, pupation, emergence and flight. Primary attractant for *Hylecoetus* in hosts, and ambrosia symbiosis in *Hylecoetus* were also studied. A review of literature pertaining to: biology; ambrosia symbiosis; and importance of *Hylecoetus* as a forest pest is given.

The life cycle was studied from 1983–1986 by following the development of immature *Hylecoetus*: (1) in naturally infested hosts in the field; (2) under controlled conditions using naturally infested pieces of wood placed in trays in a growth cabinet kept at 20°C. and 75–100% relative humidity; and (3) by keeping *Hylecoetus* on agar at 20°C. Larval instars were determined by: (1) using morphological characters distinguishing different larval instars; (2) measurements of headcapsule widths, body lengths, 'tail' lengths; the application of Dyar's rule and the plotting of frequency distributions of the measurements; and (3) by counting moulted headcapsules on agar. Pupation was observed on *Hylecoetus* kept on agar at 20°C. The larva of *Hylecoetus* is 2.38 to 25.26 mm.; the headcapsule width is 0.48 to 2.88 mm. (n=372). Winter is passed as a second, third or fourth instar larva. Most of the larvae due to pupate during the next spring become last instars before the onset of winter. Dyar's rule and frequency distributions cannot be easily applied to *Hylecoetus* due to sex differentiation by size. There may be 5 instars for female and 5 instars for male *Hylecoetus*. The pupal period is 6–8 days at 20°C. Part of the *Hylecoetus* population completes the life cycle in 1 year, whereas the rest do so in 2 years. The middle instars of *Hylecoetus* larvae (2, 3, or 4th instars) mark the longest period of development, one to two winters are passed as

these instars, whereas pupae, adults, eggs, first stage larvae and last instar larvae occur at fixed seasons within a year.

Emergence was studied from mid-April to the end of June, 1984 using naturally infested pieces of wood in the field. Emerging *Hylecoetus* were collected from cages on a daily basis. Numbers and sex ratios of emerging *Hylecoetus* were determined. Flight was investigated in 1984-1985 by monitoring flying *Hylecoetus* in the field, using standing dead larches, stumps, logs, ethanol, methanol, monoterpenes in European larch: myrcene; limonene; camphene, α-pinene; and β-pinene; and lineatin as baits. *Hylecoetus* were collected from flight traps; and *Hylecoetus* were also hand-picked from hosts, on a daily basis from 18 April to 30 June, 1984 and 8 May to 5 July, 1985; and on an hourly basis from 10.00 to 20.00 HRS on a few days with favourable weather only between 13 May and 30 June, 1984. Little or no flight data were obtained in 1985 and 1986 respectively because of poor weather conditions during the flight season.

At Dunkeld (in both Atholl and Craigvinean forests) the activity of *Hylecoetus* starts when air temperature has reached about 15°C. Emergence and flight only occur on days with favourable weather. In 1984-1985 emergence and flight occurred at Dunkeld from the end of April or the beginning of May to the end of June. Seasonal temperature, hours of sunshine and % relative humidity were the most important weather variables influencing *Hylecoetus* flight. Ambient temperature ($\geqq$15°C.) and hours of sunshine were favourable for flight, whereas high relative humidities were negatively correlated with numbers of flying *Hylecoetus*. Wind speed and rainfall also may adversely affect *Hylecoetus* flight. Interactions of weather parameters affect flight to varying degrees. The interaction of temperature and hours of sunshine is seasonally the most important trigger for *Hylecoetus* activity, whereas days with high humidities and strong winds may have the least numbers of flying

Hylecoetus or no flight at all. During the course of a day, the number of hours in which the temperature exceeded the threshold for flight (15°C.); and the temperature range above 15°C. given in degrees, are both more important correlates of *Hylecoetus* flight. Flight occurs throughout the day with a peak between 13.00 and 15.00 HRS. *Hylecoetus* flies at 2-3 m. above the ground and makes random flights. When *Hylecoetus* are in promixity to suitable hosts the flight becomes firmly directed and there is a loss in height before the eventual landing on the hosts. *Hylecoetus* approaches suitable hosts while flying head to wind and orientation to hosts seems to be guided by light and the host silhouette. Female and male *Hylecoetus* land on the same hosts for mating and oviposition. While on the hosts females with their extruded ovipositors search for suitable sites for oviposition, whereas males with their maxillary palpi expanded search for females. It is not known whether or not *Hylecoetus* attacks reduce the attractiveness of hosts to later *Hylecoetus* flights. Ethanol with or without camphene may be the primary attractant. The responce of *Hylecoetus* to ethanol may be dosage-dependent. *Hylecoetus* are capable of orienting to sources of chemical stimuli (odours) in the absence of normal visual cues. The role of ethanol may be that of a major attractant and arrestant.

The study of the biology of *Hylecoetus* included pairing or mating, oviposition, incubation, hatching and survival and development under controlled conditions. Pairing was studied in the field, and mating *Hylecoetus* were looked for in the air and on hosts; and then records of time of mating were established. In addition oviposition, incubation and hatching were observed on *Hylecoetus* kept on agar at 20°C. *H. dermestoides* exhibits sexual dimorphism, with morphological characters such as size, colour and maxillary palpi being different in the two sexes. All-black and yellowish brown males occur together. A total of 196 males were examined, 175 of which were all-black and 21

yellowish brown. The Scottish race of *Hylecoetus* have approximately a 1:1 sex ratio. Female *Hylecoetus* are significantly larger than males, being 10.3 to 16.4 mm. and 8.2 to 12.7 mm. respectively. Mating and oviposition occur throughout the flight season, and adults are short-lived (1-3 days), i.e. they die shortly after oviposition. Seven of female *Hylecoetus* from natural populations examined in 1984 laid eggs on the day they emerged, whereas 24 oviposited on the second day after emergence. Eggs were laid in batches, with batches of eggs per female ranging from 1 to 3; the first batch ranging from 4-45; the second batch 12-29 and the third batch 5-20 eggs respectively. Each female produced a total of 7-61 eggs. Female *Hylecoetus* kept on agar at 20°C. produced only one batch of eggs each, with 16-28 eggs per female. *Hylecoetus* on agar laid eggs 1-2 days after emergence. Female *Hylecoetus* reared in the laboratory seemed to be ectosymbiotic fungus-free, and these virgin females laid non-viable and fewer eggs than mated females from natural populations in the field.

Ambrosia association in *Hylecoetus* was studied by: (1) isolation and identification of the ambrosia fungi; and (2) examination of the roles of bark, wood and ambrosia fungi as sources of nutrients as determined by analyses of amino acids and sugars in larch inner bark, sapwood and *Hylecoetus* frass using chromatographic techniques, and physiological studies of ambrosia fungi. *Ceratocystis piceae* is the ambrosia fungus for the Scottish race of *H. dermestoides*. Traces of particles of wood, and fungal mycelial and spores found in the guts of *Hylecoetus* larvae indicate that the larvae feed on a wood-fungal mycelium complex. The removal of *C. piceae* from diets of *Hylecoetus* seemed to result in reductions in the numbers of eggs laid and the fertility of females or viability of eggs produced. This phenomenon does not show whether or not the symbiosis between *Hylecoetus* and *C. piceae* may be obligatory. However, the microsymbiote may be an important source of

nutrients and other growth factors necessary for growth and reproduction. The *C. piceae* infested wood has more amino acids than fresh bark and sapwod, indicating that the feeding by *Hylecoetus* on the wood-microorganismal complex may have a nutritional advantage. *Hylecoetus* acquire certain amino acids (e.g., glycine, methionine, arginine and histidine) which seem to be available in fresh bark and sapwood in smaller amounts than in the wood-fungal complex. *C. piceae* may present to *Hylecoetus* a more favourable environment in terms of nitrogen nutrition. The beneficial relationship between *Hylecoetus* and *C. piceae* seems to be the result of qualitative changes in amino acids of infested tissue. The influence of *C. piceae* seems to result in an increase in total nitrogen in the infested tissue, and this does not seem to preclude the possibility that *C. piceae* may influence *Hylecoetus* growth and development through changes in chemical compounds other than amino acids. Sucrose, glucose, fructose and raffinose are found in larch bark and sapwood. These sugars are among the components of the sugar pool that may be available to *C. piceae* and *Hylecoetus*.

C. piceae grows readily on: most of the common laboratory agar-based media; larch bark; sapwood and heartwood/agar meals sugars and amino acids in larch extracts.

The temperature of incubation influences the linear extension of *C. piceae* in culture. *C. piceae* grows at 5°-30°C.; with the most rapid extension being at 15°-25°C. Acidic medium results in more rapid linear extension, and *C. piceae* shows a wide pH-tolerance in the acid region, with the most rapid extension being at pH's 3-4, whereas pH's less than 3.4 inhibit linear extension. Nutrients, temperature and pH not only affect radial extension but also the sporulation of *C. piceae*.

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APPENDICES

Appendix 1: RELATIONSHIP BETWEEN EMERGENCE, FLIGHT, PAIRING AND OVIPOSITION IN *H. dermestoides* ON THE ATHOLL ESTATE IN 1984

Date	Numbers emerging	Flight index	Mating pairs	Ovipositing females	Number of eggs
25 April	–	0.29	0	0	–
29	–	0.08	0	1	35
30	–	0.08	0	0	–
2 May	–	0.31	0	0	–
3	0	0.23	0	0	–
13	9	2.91	4	4	–
14	3	1.30	2	5	89
15	5	0.00	0	0	151
18	1	0.00	0	0	–
19	2	0.00	0	0	–
20	1	1.35	0	0	–
21	0	0.09	0	0	–
22	0	1.17	0	1	35
23	9	1.22	0	5	–
24	1	2.00	0	5	–
25	1	0.00	0	0	–
28	0	0.80	0	5	123
31	0	1.96	1	0	–
1 June	1	0.04	0	0	–
7	10	2.94	6	4	–
8	0	0.35	0	1	130
9	0	0.00	0	0	13
12	0	0.00	0	0	59
13	4	0.80	0	1	–
14	0	0.12	0	2	–
15	2	0.69	0	2	70
16	1	0.04	0	0	43
17	0	0.00	0	0	–
18	0	0.00	0	0	–
19	0	0.00	0	0	–
20	0	0.12	0	0	–

Appendix 2: ANALYSIS OF VARIANCE:REGRESSION OF NUMBERS OF *Hylecoetus* COLLECTED FROM FLIGHT TRAPS ON DAILY EMERGENCE ON THE ATHOLL ESTATE IN 1984

Source of Variation	Df	Sum of Squares	Mean Square	Variance Ratio	Probability (P =)
Regression	1	3822.6931	3822.6931	14.1285	0.01
Residual	20	5411.3069	270.5653		
Total	21	9234.000	439.7141		

Appendix 3: ANALYSIS OF VARIANCE:REGRESSION OF *Hylecoetus* CATCH INDEX ON SINGLE WEATHER VARIABLES

Source of Variation	Df	Sum of Squares	Mean Square	Variance Ratio	Probability (P =)
Regression on wind speed	1	0.6864	0.6864	0.5495	n.s.
Residual	9	11.2426	1.2492		
Total	10	11.9290	1.1929		
Regression on % R.H.	1	5.1697	5.1679	6.8836	0.05
Residual	9	6.7592	0.7510		
Total	10	11.9290	1.1929		
Regression on Temp. (°C.)	1	5.0465	5.0465	6.5992	0.05
Residual	9	6.8824	0.7647		
Total	10	11.9290	1.1929		
Regression on Hours of sunshine	1	5.9614	5.9614	8.9908	0.05
Residual	9	5.9675	0.6631		
Total	10	11.99290	1.1929		

Appendix 4: ANALYSIS OF VARIANCE:REGRESSION OF *Hylecoetus* CATCH INDEX ON PAIRS OF WEATHER VARIABLES

No.	Source of Variation	Df	Sum of Squares	Mean Square	Variance Ratio	Probability (P =)
1	Regression on Temp.(X_1),and %R.H.(X_2)	2	8.2675	4.1337	9.0319	0.01
	Residual	8	3.6615	0.14577		
	Total	10	11.9290	1.1929		
2	Regression on Temp.(X_1) and Wind(X_3)	2	5.3433	2.6716	3.2454	n.s.
	Residual	8	6.5857	0.8232		
	Total	10	11.9290	1.1929		
3	Regression on Temp. (X_1) and hours of Sunshine (X_4)	2	8.5102	4.2551	9.9572	0.01
	Residual	8	3.4187	0.4273		
	Total	10	11.9290	1.1929		
4	Regression on %R.H. (X_2) and wind (X_3)	2	8.5134	4.2567	9.9703	0.01
	Residual	8	3.4155	0.426		
	Total	10	11.99290	1.1929		
5	Regression on %R.H.(X_2) and Hours of sunshine (X_4)	2	7.2009	3.6004	6.0920	0.05
	Residual	8	4.7281	0.5910		
	Total	10	11.9290	1.1929		
6	Regression on Wind(X_3) and Hours of sunshine	2	6.6470	3.3235	5.0338	0.05
	Residual	8	5.2819	0.6602		
	Total	10	11.9290	1.1929		

Appendix 5: SUMMARY OF REGRESSION EQUATIONS FOR RESULTS OF ANALYSIS OF VARIANCE SHOWN IN TABLE 50

Regression	Regression equation
1	$y = 0.1868X_1 - 0.0838X_2 + 1.7302$
2	$y = 0.2245X_1 - 0.2449X_3 - 2.3142$
3	$y = 0.1724X_1 - 0.1677X_4 - 3.5163$
4	$y = -0.1372X_2 - 0.8812X_3 + 9.3711$
5	$y = -0.0605X_2 + 0.1470X_4 + 2.4699$
6	$y = -0.3691X_3 + 0.2102X_4 - 0.1503$

Appendix 6: ANALYSIS OF VARIANCE:REGRESSION OF *Hylecoetus* CATCH INDEX ON TRIPLES OF WEATHER VARIABLES

No.	Source of Variation	Df	Sum of Squares	Mean Square	Variance Ratio	Probability (P =)
1	Regression on Temp.(X_1),%R.H.(X_2) and Wind (X_3)	3	10.3562	3.4521	15.3646	0.01
	Residual	7	1.5727	0.2247		
2	Regression on Temp.(X_1),%R.H.(X_2) and sunshine(X_4)	3	9.4532	3.1511	8.9092	0.01
	Residual	7	2.4758	0.3537		
3	Regression on Temp.(X_1),Wind(X_3) and sunshin(X_4)	3	8.8938	2.9646	6.8372	0.05
	Residual	7	3.0352	0.4336		
4	Regression on %R.H.(X_2),Wind(X_3) and sunshine(X_4)	3	9.4464	3.1488	8.8787	0.01
	Residual	7	2.4825	0.3546		
	Total	10	11.9290	1.1929		

Appendix 7: SUMMARY OF REGRESSION EQUATIONS FOR RESULTS OF ANALYSIS OF VARIANCE SHOWN IN TABLE 53

Regression	Regression equation
1	$y= 0.1485X_1-0.1155X_2-0.7178X_3+5.4505$
2	$y= 0.1627X_1-0.0530X_2+0.1147X_4-0.3688$
3	$y= 0.1633X_1-0.2786X_3+0.1699X_4-2.7707$
4	$y=-0.1021X_2-0.7502X_3+0.1035X_4+6.4744$

Appendix 8: ANALYSIS OF VARIANCE:MULTIPLE REGRESSION OF *Hylecoetus* CATCH INDEX ON 4 WEATHER VARIABLES

Source of Variation	Df	Sum of Squares	Mean Square	Variance Ratio	Probability (P =)
Regression on X_1,X_2,X_3,X_4	4	10.9541	2.7385	16.8540	0.01
Residual	6	0.1625			
Total	10	11.9290	1.1929		

r = 0.9583, p = 0.01; $y=0.1358X_1-0.0890X_2-0.6257_3+0.0838X_4+3.4406$

X_1=Mean daily temperature (°C.)
X_2=Mean daily % relative humidity
X_3=Mean daily relative wind speed (m/s)
X_4=Hours of sunshine.

Appendix 9: SUMMARY OF INFORMATION ON REGRESSION OF CATCH INDEX OF *Hylecoetus* ON DIFFERENT SETS OF INDEPENDENT WEATHER PARAMETERS

Variables	Temp. and %R.H.	Temp. and Wind	Temp. and Sun	%R.H. and Wind	%R.H. and Sun	Wind and Sun	Temp., %R.H., Wind	Temp., %R.H. Sun	Temp., Wind Sun	%R.H. Wind & Sun	Temp., %R.H. Wind & Sun
r	0.832	0.669	0.844	0.844	0.776	0.746	0.931	0.890	0.863	0.889	0.958
p =	0.01	n.s.	0.01	0.01	0.05	0.05	0.01	0.01	0.05	0.01	0.01

Appendix 10: EMERGENCE OF *Tomicus piniperda* (SCOLYTIDAE) RELATED TO LATITUDE OF DIFFERENT SITES IN NORWAY.

SITE	GRID REFERENCE	ELEVATION (m)	TIME OF EMERGENCE OF ADULTS
Idd	58° 06′N, 11° 28′E	180	*Tomicus* activity occurred mostly between 28 March and the first week in April.
Sögne	58° 06′N, 07° 50′E	10	
Froland	58° 27′N, 08° 37′E	50	
Fyresdal	59° 19′N, 08° 37′E	400	Most *Tomicus* activity occurred between 19 April and 13 May.
Siljan	59° 21′N, 09° 40′E	300	
Åsnes	60° 39′N, 12° 03′E	170	
Førde	61° 26′N, 05° 59′E	60	
Snåsa	64° 13′N, 12° 14′E	100	
Målsev	69° 02′N, 19° 23′E	80	Nearly all *Tomicus* were in flight between 1 and 9 June.
Sør-Varanger	69° 10′N, 29° 10′E	50	

Source: Bakke (1968).