

BEHAVIOUR OF IPS GRANDICOLLIS (EICHHOFF) (COLEOPTERA:SCOLYTIDAE)

by

Jayanthi P. Witanachchi B.Sc. (Hons.) (Ceylon)

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Department of Entomology
Waite Agricultural Research Institute
University of Adelaide

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SUMMARY

Ips grandicollis (Eichhoff) is an inhabitant of the subcortical tissues of coniferous trees, mainly Pinus species. (1)

It has been classed as a pest of secondary importance, with the potential to become a primary pest under outbreak situations, both in its native habitat in North America and in South and Western Australia (where it became established in about 1943).

Investigations carried out during this study have been restricted to its major host species in Australia; Pinus radiata D. Don. Factors which contribute to resistance of individual trees to attack by I. grandicollis were investigated. The defense mechanism usually involves the flow of resin from reservoirs and ducts damaged by beetles during initial boring. Failure to encounter any of these resin barriers, cessation of boring and retreat was associated with secondary resin produced by the damaged phloem cells. The absence of such defence mechanisms in declining trees, [♀] resulted in continued boring. ✓

During Dispersal, I. grandicollis was found to land randomly (2) on trees within a pine stand, the actual selection being made after boring into the bark of the tree.

Dispersing adult I. grandicollis, either caught on pheromone-baited traps in the field or collected in the laboratory during emergence from field infested logs, included a high percentage of (3) mated females. Mating occurred only within galleries in the bark. Some of the progeny adults, including siblings, mated before emergence and later produced offspring. The number of females

thus mated depended upon the time spent within the bark by mature adults before emergence from the host in which they developed. Virgin and mated females initiate galleries even in the presence of male-initiated galleries. Moreover, males join both virgin and mated females which have produced galleries. Similarly, both virgin and mated females were accepted into nuptial chambers by males.

A technique using GC/MS was developed to determine the concentration of ipsenol in extracts of whole beetles. Production of ipsenol in males began about 9 to 12 hr after boring into suitable host material and this tended to coincide with the appearance of faecal pellets in the frass. Adult males which rejected bore-sites in 'resistant' trees had not produced ipsenol. These data indicate that males do not feed and hence do not produce any ipsenol until the decision to continue boring has been made. This evidence supports the generally accepted concepts of ipsenol production and its dependence on feeding by the male, but it provides a more critical basis for the development of secondary attraction of I. grandicollis than has so far been presented.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and, to the best of my knowledge and belief, contains no material previously published or written by another person, except when due reference is made in the text.

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Jayanthi P. Witanachchi

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SECTION 1

INTRODUCTION

1. INTRODUCTION

1.1. Ips species as worldwide pests of conifers

Bark beetles of the genus Ips DeGeer (Coleoptera : Scolytidae) are inhabitants of the subcortical tissues of coniferous trees, predominantly pines and spruces. This genus is most strongly represented in the Palearctic and Nearctic regions of the world, with 7 Eurasian and 32 Nearctic species (Hopping, 1963). (1) (5)

The economic status of the species of Ips bark beetles has never been definitely established. Of the 30-odd species in this genus, few are recorded as aggressive primary pests of conifers. The question of whether or not these bark beetles are able to attack completely healthy trees, thereby qualifying as primary pest species has been a subject of repeated discussion among forest entomologists over the last century. Literature on the destructiveness of Ips species is somewhat variable, since trees that had been subjected to natural attack, cannot be detected until after the attack is well under way.

Hopkins (1899) was one of the first to describe some of the Ips species as dangerous secondarily destructive pests of conifers. Swaine (1918) held the same view, but recognised the ability of Ips calligraphus to become a primary invader. Dodge (1938) also considered the same species to be a dangerous primary pest. Doane et al (1936) stated that although most members of the genus Ips are secondarily destructive species, they may attack adjacent healthy trees once the population had built up in

suitable breeding material. The same authors speculated that with the removal of virgin forests, Ips could be a greater threat to second growth pine stands.

Chamberlin (1939) attempted to divide the family Scolytidae into groups according to their relative economic importance. His first group included primary pests, or those that caused the death of living trees. In this category he placed certain species of Dendroctonus and a few species of Ips. However, the author fails to mention evidence upon which any species of Ips had been accorded primary pest-status. His second group included the majority of Ips species that breed in suppressed or weakened trees and in slash.

Following World War II, several severe outbreaks of bark beetles occurred in Europe due to poor forest management. This provided the stimulus for intensive studies, which resulted in a burgeoning of relevant literature that Rudinsky (1962) has reviewed. Studies in the last five years have been of immense importance and include investigations into the effects of the physiological condition of host-tree upon the population dynamics of bark beetles. Based upon results of these studies, an hypothesis, currently accepted by most forest entomologists, emerged; that both the physiological condition of the host tree and the population level of the insect, together determine the degree of destructiveness of any bark beetle species. This thesis, supported by much data from various parts of the world, relates the pest status of the bark beetle to its population density and the susceptibility of the trees being attacked by that population. Hence any attempt to group the species of Ips

according to their destructiveness is meaningless unless the conditions under which successful invasions occur are closely identified.

Based on Kéler's (1956) definition of primary pests, "those that prefer completely healthy trees of full vigour", all or most of the Ips species must be considered as secondary pests. Rudinsky (1962) clarifies the term 'to become a primary pest' commonly used in forest entomology, as the temporary ability of bark beetles to kill healthy trees compared with the constant 'preference' to breed in such trees by primary pest species, as defined above. Based on such definitions, the term 'secondary pest' does not imply any economic loss, only the ability to breed in temporarily or permanently weakened trees (Zwolfer, 1957).

A survey of the literature with a view to finding out the destructiveness of Ips species has indicated that only two species qualify as primary pests, based on currently accepted definitions. These are : the Nearctic I. calligraphus (Germ.) as described by Swaine (1918) and Dodge (1938), and the Eurasian I. typographus (Linn.), so defined by Keen (1952) and Schwerdtfeger (1955). Several other species of Ips have been described as secondary pests under 'normal conditions' but each with the ability to become a primary pest under outbreak situations. Ips. emarginatus(Lec.), (Chamberlin, 1939; Keen 1938), I. integer(Eichh.) (Swaine, 1918; Keen, 1938), I. confusus (Lec.) (Chamberlin, 1939; Keen, 1938), I. perturbatus(Eichh.) (Swaine, 1918), I. pini(Say), (Swaine, 1918), I. oregoni (Eichh.) (Swaine, 1918; Keen, 1938; Chamberlin, 1939), I. lecontei Sw. (Chamberlin, 1939), I. avulsus (Eichh.) (Chamberlin, 1939),

I. radiatae Hopk. (Keen, 1938; Chamberlin, 1939) fall into this category. Almost all of the species of Ips occurring in Eurasia have been placed in this category by Lekander et al (1977). The rest of the species of Ips that have not been mentioned here, have been described either as secondary pests or as neutral species that are not known to cause any injury to living trees (Swaine, 1918).

In conclusion one must agree with the recent findings of Lekander et al (1977), that not all of the species of Ips have earned pest-status and that, in many cases, it is not possible to predict accurately the potential importance of such species to man or to the ecosystem.

1.2. Status of Ips grandicollis as a pest

Ips grandicollis which is indigenous to North America, occurs from Quebec to Florida in the east and from Manitoba to Texas in the western part of its range (Hopping, 1965). It became established in South Australia in 1943 (Swan 1943; Swan, 1950) and later in Western Australia (Rimes, 1959) and in Cuba in 1970 (Nunberg, 1974).

Its known hosts in North America are Pinus echinata Mill., P. taeda L., P. sylvestris L., P. virginiana Mill., P. palustris Mill., P. rigida Mill., P. caribaea Morelet, P. banksiana Lamb., P. strobus L., (Hopping, 1965). In Australia its known hosts are P. muricata D. Don., P. radiata D. Don., P. halepensis Mill., P. canariensis C. Smith., P. nigra calabrica Arn. (Loud.) Schneid. P. pinaster Ait., (Morgan, 1967). In Cuba its only known host is P. tropicalis (Morelet) (Nunberg, 1974).

Also slash, sand, and pond pines in Fla. (See Little's Checklist of US Trees) write New Edition just out.
 NGS: P. caribaea = P. elliottii in U.S. may be o.k. for Central Am.

Reports and records on the pest-status of I. grandicollis have been very variable. This perhaps may be due to the difficulty in assessing the ability of I. grandicollis to colonise healthy trees, or due to the variability in the behavioural characteristics of this species in different host species, as well as under different climatic conditions.

Hetrick (1942) reporting on the bark beetle situation in East Virginia, rated I. grandicollis as of secondary importance. Here it is found with two other species, I. avulsus and I. calligraphus, which, under outbreak conditions, are responsible for primary attack on large loblolly pines (P. taeda). Rennels (1962) described how healthy red pine (P. resinosa Aitkins) 6 to 14 ft high, were successfully attacked by I. grandicollis adults emerging from logging debris in Illinois. He considered this beetle a serious problem in pine stands of declining vigour due either to competition in unthinned stands or to prolonged drought. Mason (1969a) on the other hand, recorded the behavioural characteristics of this species, in particular its slow dispersal after emergence from the host, because of which the beetle tends to linger in the former thinning leading to reinfestation of old slash. He therefore concluded that I. grandicollis seldom attempts to invade healthy trees, on which its few attacks usually fail. Baker (1972) reported that I. grandicollis appears to prefer felled trees and slash usually, but that trunks and limbs of apparently healthy trees have also been subjected to heavy attacks. Moreover, spot or group killing of pines has been observed to be a characteristic of this species, especially during periods of extreme droughts when its population numbers increase. On the other hand, Hain and Anderson (1976)

observed that recently emerged I. grandicollis beetles dispersed before initiating attacks in new hosts, thereby preventing excessive concentration of beetles in a small area.

In Australia, I. grandicollis was first recorded from P. laricio at Wirrabarra forest in South Australia (Swan and Borden, 1943; Swan, 1950; Brimblecombe, 1953). It has since become established in Western Australia (Harding, 1952; Rimes, 1959).

In South Australia there is very little evidence of successful attack by I. grandicollis on living trees, except for a specific type of attack that occurs occasionally (Morgan, per comm.). These attacks caused death of trees in young plantations specifically. Morgan (1967) described such attacks as 'feeding attacks' in comparison with the usual breeding attacks that occur in slash or in suppressed trees. During such 'feeding attacks', the characteristic type of galleries constructed by breeding adults were not observed; instead the outer bark was seen to fall away in large patches due to feeding on the underlying inner bark. In standing trees, however, the usual type of breeding attack has been confined to suppressed trees of low vigour. Hence the economic loss associated with I. grandicollis has been considered insignificant to the forest industry.

In Western Australia too, I. grandicollis is not considered a serious pest of pines. Although there is no evidence of it killing live trees, there is a suspicion that the so called 'lime-stone deaths' have been aggravated by I. grandicollis attack, when trees are at wilting point. Such trees may have

recovered had there been no beetles to attack them.

In Cuba when I. grandicollis was first found in 1970, it was considered to be a serious pest of P. tropicalis (Nunberg, 1974) but no further information is available.

Although I. grandicollis is considered a secondary pest in most circumstances because it rarely attacks living healthy trees, it could cause severe damage to the lumber industry. Wood staining fungi, Ceratocystis ips Rumbold and C. minor (7) (Hedge.), have been found in close association with Ips grandicollis and its galleries, especially in Pinus radiata (Vaartaja, 1967). The blue staining of the sapwood due to the activity of these fungi, causes deterioration of the quality (8) of timber and hence is of economic importance to the lumber industry.

The association between the fungi and I. grandicollis is of further importance for two reasons. Mathre (1964) has shown that C. minor can kill small ponderosa pine trees, 10 to 15 years old, within 15 to 22 days from inoculation. (9) Secondly, C. minor is found in constant association with certain species of bark beetles (Mathiesen, 1950). As a result many workers have suggested that this fungus may play a role in the death of trees attacked by beetles.

The act of boring into the cambium-sapwood interface by bark beetles, followed by construction of vertical galleries along the bole of host trees is considered similar to the girdling of trees by mechanical action. However, as mechanically

girdled trees may live a few years, the girdling action of the beetles alone does not explain the resulting rapid death of trees.

Nevertheless, the question of whether the blue-staining fungus associated with I. grandicollis is pathogenic to the host tree has not been satisfactorily answered. (Yet its effect as a wood-deteriorating factor is not in doubt).

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In conclusion, it should be mentioned that I. grandicollis is generally considered a pest of secondary importance, throughout its present distribution. But like most other bark beetle species, it has the potential to become a primary invader, either under outbreak situations (Baker, 1972), or in pine stands of declining vigour (Rennels, 1962) or in very young plantations when concentrations of active beetles occur in a locality (Morgan, 1967). However, the indirect damage caused by the fungal associates of which I. grandicollis is a vector, could be of importance to the timber industry.

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1.3. Biology and behaviour of I. grandicollis

1.3.1. Classification

The taxonomic status of I. grandicollis was questioned by Schedl (1955) who was of the opinion that I. chagnoni attacking white spruce in Canada was very similar to I. grandicollis. Subsequently, Hopping (1965) confirmed that I. grandicollis and I. chagnoni are conspecific and placed chagnoni as a junior synonym of grandicollis. He further pointed out the great variability in size of I. grandicollis individuals; those

from the southern part of its range being smaller than those from the northern areas, with a progressive gradation in size from north to south.

Hopping (1963a) classed the genera Pityokteines Fuchs, Orthotomicus Ferrari, Orthotomides Wood, together with Ips DeGeer in the Sub-tribe Ipina, of the Tribe Ipinini under the Sub-family Ipininae of the Family Scolytidae. Hopping (1963b) placed the 32 species of Ips recognised in North America into 10 groups, based on the characteristics of the declivital spines, sutures of the antennal club and the male genitalia. I. grandicollis has been placed in Group IX of Hopping's classification, together with four other species, I. confusus (Leconte) I. montanus (Eichh.) I. cribricollis (Eichh.) and I. lecontei Swaine, on the basis of the presence of 5 spines on each lateral margin of the posterior elytral declivity. However, the characteristic nature of the elytral interspaces (impunctate on the disc) of I. grandicollis, distinguishes it from the other four species, in the same grouping (Hopping, 1965).

1.3.2. Past work

Although much work has been done on most of the species of Ips of North America, I. grandicollis has attracted relatively little attention. This is mainly because it usually occurs with more economically important destructive species. Hence the paucity of literature on this species.

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Haliburton (1943) studied the biology and life history of I. grandicollis in its indigenous habitat. Bungey (1966) and Morgan (1967) studied the biology and behaviour of this species under Australian climatic conditions.

Morgan (1967) outlined the biology and behaviour of I. grandicollis based on studies on natural infestations in South Australia, while Bungey (1966) described the biology, behaviour and chemical control of I. grandicollis based on field and laboratory studies.

In the past decade interest has been focussed mostly on behavioural studies relating to the attack pattern of I. grandicollis. The work of Mason (1969), Berisford and Franklin (1969), Berisford and Franklin (1971), All and Anderson (1972), deal with this aspect of behaviour. On the other hand the work of Wilkinson (1962) and Wilkinson et al (1967) relate to the importance of stridulation on the behaviour of I. grandicollis.

Following such studies, in the second half of the past decade in particular, interest shifted to insect- and host-produced attractants that govern the behaviour of I. grandicollis. Studies by Wilkinson (1964), Morgan (1967), Hertel et al (1969) and Werner (1972b) indicated that I. grandicollis adults aggregate on Pinus species in response to attractants released by males feeding on host material. Investigations by Vité et al. (1964) and Vité and Pitman (1967) showed that the insect-produced attractant is generated in the hind gut and released by defaecation. They also showed that the attraction of I. grandicollis occurs

in response to host oleoresins. Isolation and identification of the male-produced population aggregation pheromone by Vité and Renwick (1971), was a major advance in studies on the behaviour of I. grandicollis. Following this discovery, Werner (1972 a,b,c), Vité et al (1972), Hughes (1974), Brand et al (1975) and Vité et al (1976) investigated the various aspects of production, synthesis and response to this attractant.

1.3.3. Behaviour

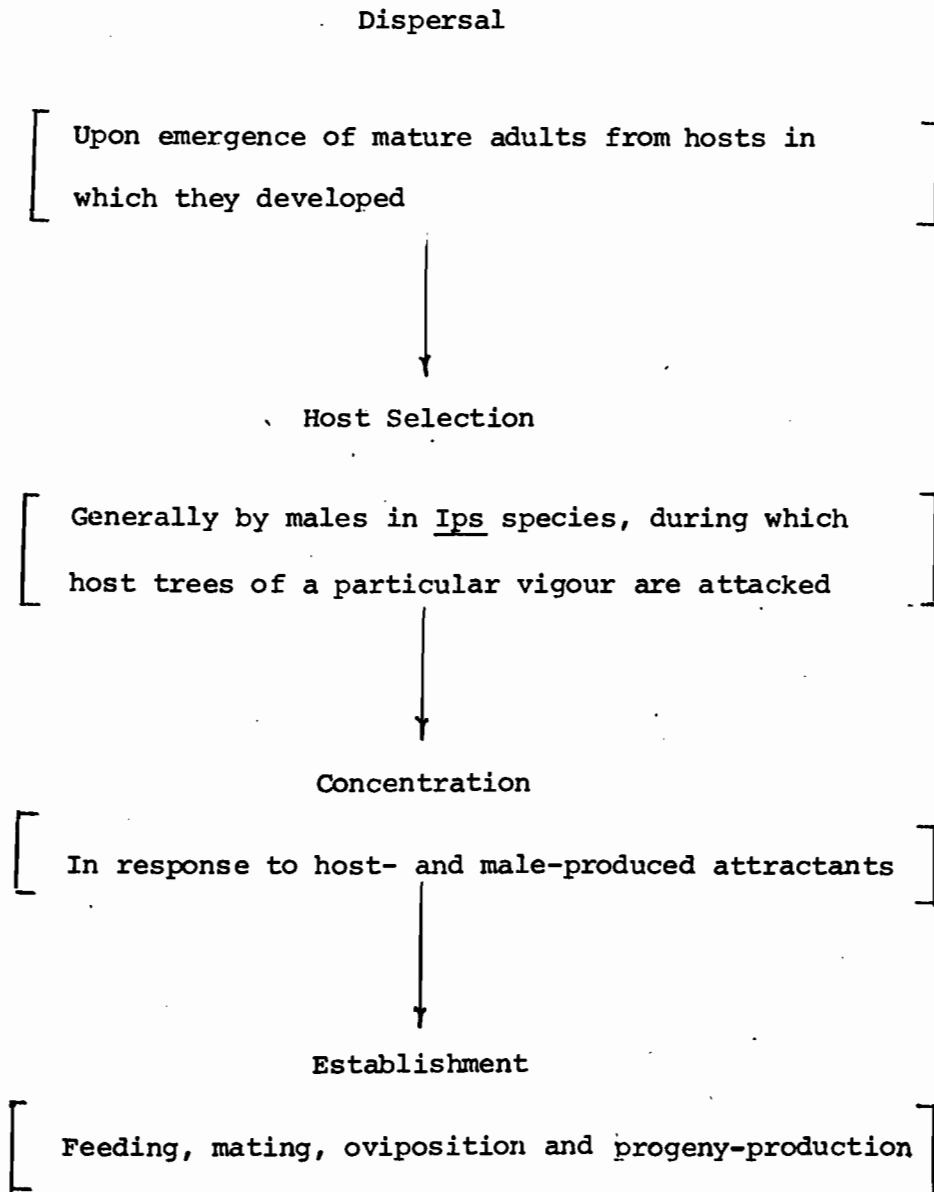
Attempts to describe the complex adult behaviour pattern of bark beetles, have led to the system adopted by Cobb et al (1968). This system suggests three phases: dispersal, concentration and establishment when describing the adult behaviour pattern of most bark beetles.

The system is outlined in Figure 1, except that host selection has been considered as a separate phase from that of dispersal.

The dispersal phase begins with the emergence of the adults from the host in which they developed.

Emergence of these individuals refers to the act of leaving the bark of the host in which they developed, after which the beetles usually take to flying. Similarly, dispersal refers to the act of flying, during which the beetles may encounter hosts on which they may land and into which they may begin to bore. ^{In S. Austral.)} Emergence and dispersal of I. grandicollis occur during the period from mid-October till late April or early May, reaching a

Fig. 1 Generalised sequence of behaviour of Ips bark beetles (after Cobb et al 1968)



peak in early February (Morgan, 1967). Emergence of bark beetles is governed by the ambient temperature (with significant periods >22°C) that is prevalent from October to April for most years in South Australia (Anon. 1978).

Following emergence the adults disperse and may encounter new host material (i.e. they enter the phase of host selection).

During this process males locate their host tree, which in South Australia is mainly P. radiata. Wood (1972) has reviewed most of the early theories on the host specificity of bark beetles. Also, it has been shown by Hodges et al (1979) that differences in susceptibility of host species to specific bark beetles are possibly due to the differences in physical and chemical properties of their oleoresins.

With respect to the act of discrimination of individual trees within a host species, two hypotheses have been put forward.

Person (1931) suggested that bark beetles are capable of discrimination between a 'resistant' tree and a 'susceptible' tree in response to olfactory cues from the 'susceptible' trees.

Callaham (1952 in Wood 1972) on the other hand proposed that host-selection is a random process where the success or failure of the attack is determined by the quantitative resin flow from the attacked tree; after the beetle has initially bored into it.

Hitherto no study has been carried out to test which of these two

hypotheses hold true for Ips grandicollis. Nevertheless, in

Ips grandicollis, continuous boring after landing on the host

OK.

doubtful

(12)

species, is an indication of successful host-selection, whereas little boring and emergence from attack sites is an indication of host resistance to attack.

Following continuous boring by the host-selecting beetles; usually the males in all polygamous Ips species, the phase of population concentration begins. This occurs in response to both male and host-produced attractants (Wilkinson, 1964; Morgan, 1967; Hertel et al 1969; Vité and Renwick, 1971; Werner, 1972 a,b,c). This initial attraction is supposed to lead to subsequent mass-attraction, as in other bark beetles.

The third phase; phase of establishment includes mating, construction of egg galleries and oviposition in suitable hosts. Mating has been known to take place soon after the entry of the female into galleries or nuptial chambers excavated by males. Male I. grandicollis have been observed to take from 40 to 72 hr to construct a nuptial chamber in suitable hosts (Morgan, 1967). Observations by Wilkinson (1962) ^{et al (1967)} indicated that males ^{Ips calligraphus} accept females into nuptial chambers, only if they stridulate at the gallery entrance. Males, being polygamous, have frequently been observed with 3 to 5 females. After mating, each female begins to construct an egg gallery that radiates from the central nuptial chamber. During this process, frequent acts of copulation have been observed (Bungey, 1966). Eggs are deposited in niches, alternately carved on either side of the female gallery.

Like all other bark beetles, females of I. grandicollis are known to produce more than one batch of eggs in their life-time, often in a number of host trees. After producing one or more batches

of eggs in a particular host, a female sometimes re-emerges and re-enters another host for further egg production. This usually happens when the host in which she produced her first batch of eggs becomes unsuitable for further oviposition, either through deterioration of the bark or through the density of attack upon it. Such females may initiate their own galleries (All and Anderson, 1972) or enter a nuptial gallery constructed by a male, where she may mate several times, during the process of constructing an egg gallery. Whether such matings are a prerequisite for further oviposition, after the first mating, has not been confirmed. However, female bark beetles belonging to the genus Dendroctonus do not require further mating to establish their second batch of eggs (Reid, 1958).

1.3.4. Life cycle

Egg - The eggs are 0.75 to 0.8 mm long and 0.5 mm wide. They are globular to ovoid in shape and white. Field studies have indicated a mean number of 49 eggs per gallery per female (Morgan, 1967). Bungey (1966) recorded a maximum of 91 eggs per female per gallery. As females may produce more than one batch of eggs in a number of trees, the actual fecundity has not been (13) determined.

The eggs hatch in about 8 to 12 days at 20°C, but under (14) field conditions in mid-summer, eggs may hatch in as little as 5 days (Bungey, 1966).

Larva - The larvae are whitish, soft bodied, segmented legless grubs. They have a prominent head capsule with hypognathous mouth parts. They tunnel at right angles to the egg gallery and feed upon the inner bark as well as the fungal flora under bark. The larvae go through four instars before pupation.

Larvae require 36 days to reach the final instar at 20°C, (14) but only 15 to 20 days under field conditions in mid-summer (Bungey, 1966). The fourth-instar larva constructs an enlarged chamber at the end of its larval gallery, where it pupates.

Pupa - The soft bodied, whitish exarate pupa lying in the pupal chamber is about 4.2 mm long and 1.5 mm wide. Disturbed pupae occasionally make circular movements with their abdomens. Development of the pupae to adult eclosion takes (14) 8 to 9 days at 20°C, but only 4 to 5 days under summer conditions in the field (Bungey, 1966).

Adult - The callow adults that eclose from the pupae, (14) undergo sclerotization in 7 to 9 days at 20°C. During the same period, the adults undergo a change in colour associated with maturation. The pale brown teneral adult goes through shades of reddish-brown to attain the final chocolate-brown colour of the mature adult. The adults feed on the inner bark during this period (maturation feeding), after which they emerge by boring their way through the bark.

The adult I. grandicollis varies, in length from 3 to 4 mm; in width from 1 to 1.3 mm and in colour from reddish-brown to black. ~~The sexes~~ can be distinguished by the morphological characters of ~~the femora~~ (Wilkinson, 1962), while ^T the presence of the stridulatory organ in the female clearly distinguishes her from the male. ^L The males tend to have a coarser frontal tubercle than the females. ✓

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1.3.5. Biology

During the emergence and dispersal period, that extends from late spring to early autumn, 4 to 5 generations are produced in South Australia. The generation-time varies with the ambient temperature, thereby leading to an overlap of generations. Also, the females tend to lay their eggs over a period of time as a result of which the emergence of a generation extends over a period of 3 to 4 weeks.

The generation that develops through winter, from eggs laid in late autumn takes about 6 months to adult emergence as the larvae, pupae and adults over winter. The period from maturation to emergence is also longer for this generation. The adults of this winter generation, together with the parent adults that over-wintered, emerge from mid-October to late November. As the breeding season advances, generations develop at a much faster rate, taking about 4 to 6 weeks from egg to adult emergence. Development of the first summer generation, from

eggs laid by the adults of the winter generation, continues until January. The period spent under bark by this generation is relatively short as the adults emerge soon after maturation. The second and third summer generations develop faster, with emergence and dispersal of the adults taking place in late February and late March respectively. The earlier emerging adults of the fourth generation may either produce a fifth generation that will over-winter as larvae and pupae, or the adults may simply over-winter without ovipositing. The later developed adults of the fourth generation may over-winter in the host in which they developed, to emerge later with the fifth generation that develops through winter.

1.3.6. Factors contributing to mortality

High mortality has been observed specially in the over-wintering generation, the mean percentage being 42 (Bungey, 1966). The winter rains in South Australia make the under bark habitat water-logged, thereby subjecting the over-wintering forms to fungal and bacterial attack. This is a major factor contributing to annual beetle mortality. The over-wintering population may also be subjected to parasitism by nematodes and mites. Predation by birds is another factor contributing towards reduction in numbers of the winter generation.

Chamberlin (1939) lists two hymenopterous parasites of I. grandicollis from America, Spathius canadensis Ashm. (F. Braconidae) and Ceciclostiba dendroctoni Ashm. (F.

Chalcidoidea), while Berisford et al (1971) recorded nine species of parasites belonging to three other families, Eurytomidae, Pteromalidae and Torymidae. So far no hymenopterous parasites of I. grandicollis have been described from Australia. (16)

1.3.7. Associates of I. grandicollis

Bungey (1966) has carried out a survey of the living organisms associated with I. grandicollis. Of the fungal associates Ceratocystis ips (Rumbold) ^{C. A. R. ...} is of much economic importance as a wood-staining organism. Other fungi such as C. macrophoma, Aureobasidium spp. and Penicillium spp., have been observed by Vaartaja (1967). The nematode Contortylenchus grandicollis Massey is found in very large numbers in the body cavity and alimentary tract of some adult beetles. From exclusion experiments and successive rearings of generations of Ips grandicollis in artificial media (per. comm. Johnston) (8)

concluded that this nematode has little effect on the survival, and the fecundity of adult I. grandicollis. He further found that fungivorous nematodes (family Rhabditidae) occupy bark-beetle galleries and are also carried on the abdominal tergites of dispersing adults. They are considered to be commensals.

The mite Macrocheles merdarius Berlese, is found often in large numbers in the galleries and on the bodies of adult I. grandicollis.

Two other species of bark-beetles, Hylastes aster Paykull and Hylurgus ligniperda F., infest Pinus slash. Both species usually infest slash that is closely associated with the ground, which has been cut about 6 to 8 weeks. Hence they tend to attack slash at a later stage than does I. grandicollis. Also, Hylastes and Hylurgus tend to restrict themselves to stumps and roots that are below ground level; areas of host trees seldom colonised by Ips grandicollis. Hence these two species of scolytids seldom compete with I. grandicollis in South Australia.

1.4 Scope of study

Field and laboratory studies were carried out in South Australia to obtain more information on the interaction between Ips grandicollis and its host, Pinus radiata, as well as on certain aspects of intraspecific behaviour of this bark beetle.

The specific objectives were :

- a) to test the theories on host selection by bark beetles put forward by Person (1931) and Callaham (1952 in Wood, 1972) (17)
- b) to investigate the mechanism by which I. grandicollis locates suitable hosts and differentiates between it and other host material;
- c) to determine the specific factors within a host that contribute to its resistance to the establishment of I. grandicollis;
- d) to examine mating behaviour and to define the roles of the sexes in initiating galleries in the light of speculations of previous workers on these activities (Wilkinson, 1964; All and Anderson, 1972);
- e) to study some of the conditions under which the aggregation pheromone, ipsenol is produced and to relate these to behavioural characteristics of adults, specifically during the selection of suitable trees on which to feed and breed .

SECTION 2

PROCESS OF HOST SELECTION IN IPS GRANDICOLLIS

2. PROCESS OF HOST SELECTION IN IPS GRANDICOLLIS

BASED ON THE THEORIES OF PERSON (1931) AND CALLAHAM (1952 IN WOOD, 1972)

2.1. Introduction

Most bark beetles are known to be able to establish in trees, within their host range that are in a particular state of vigour. This ability of the scolytids to colonise and establish in particular trees has long been recognised as a vital factor in the prediction of bark beetle outbreaks. It is important also in devising preventive controls to understand the behaviour leading to successful colonisation and establishment of those bark beetle species that are known to attack any living host tree (Miller and Keen, 1960).

Upon emergence from the host in which they developed, bark beetles disperse and may subsequently encounter a new host tree. During this process of host finding, bark beetles often locate their host species from the many other tree species growing in mixed species stands.

Hopkins (1902) attempted to explain the specificity of bark beetles for particular hosts and the resistance of certain individual trees, to their attack on the basis of the interaction between Dendroctonus ponderosae and its hosts, the Pinus ponderosa complex. Callaham and Miller (1952 in Wood, 1972) theorised that the major factor governing host specificity, is the ability of a specific bark beetle to tolerate the production of oleoresin by host species. Smith (1966) supported

(18)

Callaham's hypothesis by showing reduced feeding and high mortality of D. brevicomis in the presence of oleoresin of non-host species among several other tree families. Wood (1963), Stark (1965) and Smith (1966) have reviewed most of the early unpublished research on this subject and more recently Hodges et al (1979), attributed differences in susceptibility among host-tree species to differences in the physical and chemical properties of their oleoresin. Such investigations led to a further look at Person's (1931) theory on the ability of D. brevicomis to discriminate between individual ponderosa pines in terms of resistant and non-resistant trees.

2.2. Theories

According to Person's (1931) theory, the initial attraction of bark beetles to susceptible trees is a directed response to host odours from trees of subnormal vigour, as a result of abnormal enzyme activity within their inner bark. A competing theory by Callaham (1952 in Wood, 1972) regarding individual tree discrimination within a given host species, states that, following random landing (2) of the dispersing beetles and subsequent to their initial boring activity, the quantitative resin flow from the attacked sites, determines the success or failure of the attack.

Although both theories attempt to describe how bark beetles bring about a second level of discrimination (i.e. distinguish

between resistant and susceptible trees within their host species) it is difficult to draw an absolute distinction between the two theories. However, it is clear that Person's (1931) theory involves a directed response that leads to landing of the dispersing beetles on the appropriate host tree, whilst Callaham's theory involves random landing on any tree followed by post-attack effects that subsequently lead to acceptance or rejection of a tree.

However, neither of these two general theories have been experimentally supported to the extent that either can be discarded in favour of the other. Hence, both remain a subject of much debate. Each seems to be based to some extent, on a specific bark beetle-host situation, and apparently involves olfactory, visual and other behavioural mechanisms.

In the study reported in this section, an attempt has been made to explain the process of individual tree discrimination, from a study of the inter-relationships between I. grandicollis and one of its host species, P. radiata. However, the factors which govern the first level of discrimination (i.e. host specificity) is not investigated in this study.

Ips grandicollis breeds in trees of declining vigour or in slash of P. radiata. Whether it selects such trees from healthy trees by a directed process as suggested by Person (1931) or by a random process followed by post-attack effects as stated by Callaham is tested in the experiment that follows. The experiment was based on the hypothesis of Person (1931) that

dispersing I. grandicollis beetles do initially discriminate between susceptible and resistant trees prior to landing.

2.3. Methods and materials

The study was carried out in an infested pine plantation at Mount Crawford 60 km N.E. of Adelaide, South Australia, during the summer months (November, December) of 1976. The study included an initial selection of trees based on a 'risk-rating' system for natural attack by I. grandicollis. From the many criteria of resistance of P. radiata, the colour of the crown foliage as well as the condition of the inner bark of trees (Mason, 1966), were used in applying a risk-rating to trees. The bark of 'susceptible' trees when stripped between phloem and outer bark showed no indication of secondary resin production and their foliage was yellowing. Trees with green crowns and inner bark which produced secondary resin on injury were classed as resistant trees.

Forty trees, twenty from each of the risk categories, situated at intervals of 50 m, were selected for the study. The selected trees whose d.b.h. ranged from 21.3 cm to 32.0 cm were in a 30-year old plantation.

In order to catch any beetles that would land on the test trees Tangle-foot[®] coated mesh traps (35 x 70 cm), were hung close to the bole and below the first living branch of each tree. Of the 20 trees in each category, 10 were randomly selected as controls, and no attractant was placed on their traps. The

remaining traps from each category of trees, were baited with 0.1 ml of the aggregating pheromone, ipsenol (Vité and Renwick, 1971). The pheromone was located at the centre of the trap, in a glass vial with a perforated cap. Field observations indicated that I. grandicollis flew directly to ipsenol baits when coming within 50 to 100 m of them (Morgan per. comm.). Therefore, traps were placed in such a way that trees with control traps were ca. 100 m away from those with baited traps. This arrangement ensured that none of the beetles on being attracted to baited traps would land on trees with control traps. All traps were monitored over a period of 4 weeks, during which the beetles that landed on each trap were counted and sexed. For the first 4 days traps were (20) monitored daily, thereafter twice weekly. The pheromone baits were renewed every two weeks.

2.4. Results

No significant difference in numbers of beetles landing on control traps held on susceptible trees and resistant trees were observed (Table 2.1). This equality was evident from the data obtained both during the initial four day collections and from the subsequent catches, and was therefore consistent for the entire period of the experiment. No significant difference in numbers of beetles caught on baited traps held on resistant and susceptible trees were observed during the initial 2 to 3 day period. But from the third day onwards, the baited traps on susceptible trees caught a significantly greater number of beetles than the ones on the resistant trees (Tables 2.2 and 2.1).

Table 2.1

Numbers of dispersing I. grandicollis beetles caught over a period of four weeks on control and baited traps held on 'susceptible' and 'resistant' P. radiata trees. (Total number of traps = 40)

No. & type of trap	Nos. caught/trap on 'susceptible' trees $\bar{x} \pm \text{S.E.}$	Nos. caught/trap on 'resistant' trees $\bar{x} \pm \text{S.E.}$
20 Control traps	11.3 \pm 1.0 (a)	10.3 \pm 0.9 (a)
20 Baited traps	1778.7 \pm 80.64 (b)	800.6 \pm 29.89 (c)

(20)

Mean values followed by different letters differ significantly at P = 0.05.

Table 2.2

Numbers of dispersing I. grandicollis beetles caught over varying periods on baited traps held on P. radiata trees, risk-rated 'susceptible' and 'resistant'. (Total of 10 traps for each treatment)

Duration of catches (days)	Nos. caught/trap on 'susceptible' trees $\bar{x} \pm S.E.$	Nos. caught/trap on 'resistant' trees $\bar{x} \pm S.E.$
D ₀ - D ₁	37.7 ± 2.9	39.6 ± 3.7
D ₁ - D ₂	31.7 ± 5.6	28.6 ± 5.4
D ₂ - D ₃	27.8 ± 4.0	25.7 ± 2.3
D ₄ -D ₇	195.9 ± 2.7	84.4 ± 5.3
D ₈ -D ₁₄	448.2 ± 4.9	187.0 ± 2.4
D ₁₅ -D ₂₁	492.6 ± 8.0	206.4 ± 4.7
D ₂₂ -D ₂₈	544.8 ± 6.4	228.6 ± 2.0

(20)

Of the beetles attracted to ipsenol baited traps, some that had landed on the boles of the test trees were observed to be boring into the bark. Further observations indicated a distinct difference in their behaviour depending upon the risk-rating of the tree. Individuals landing on trees rated 'susceptible', bored their way into and established themselves by constructing verticle galleries within the inner bark. There was no resin flow associated with these galleries. Those landing on 'resistant' trees bored only up to the inner bark and then either retreated or were drowned in the resin that exuded from almost all the entry holes bored in such trees.

No beetles were observed to have landed or bored into either category of trees that had the control traps on them. This could be expected as few beetles were caught on control traps (about 10 beetles/trap as against more than 800 beetles/ipsenol baited trap) over the same four week period (Table 2.1).

One week after the completion of the initial experiment, the area under the bark at some of the entry holes made both in 'susceptible' and 'resistant' trees were examined. The results (Table 2.3) indicate an absence of any vertical galleries in trees risk-rated resistant, and the presence of short galleries that ran radial or horizontal to the bole of the tree, and reached (21) a maximum depth to the phloem sapwood interface. These galleries had been either abandoned or had dead beetles trapped in their resin. In trees risk-rated 'susceptible', vertical galleries reaching a mean length of 36.5 cm had been constructed. The presence of a mean number of 29.2 eggs/gallery in certain of the galleries, indicated successful establishment of beetles in such trees.

Table 2.3

Nature of the area under bark at some of the sites with entrance galleries of I. grandicollis in 'susceptible' and 'resistant' P. radiata trees bearing baited traps. (Number of trees observed in each category = 5. Number of galleries examined per tree = 10)

Host type	Maximum gallery length (cm) $\bar{x} \pm \text{S.E.}$	No. eggs/ gallery $\bar{x} \pm \text{S.E.}$
'Resistant'	0.34 \pm 0.02 *	0
'Susceptible'	36.5 \pm 2.7 **	29.2 \pm 2.1

*Horizontal galleries reaching mainly the inner bark

**Vertical galleries made along the phloem-sapwood interface.

2.5. Discussion and conclusion

As equal numbers of dispersing beetles landed on control traps held on both susceptible and resistant trees, it may be inferred that the dispersing beetles do not discriminate between resistant and susceptible trees prior to landing. Hence, Person's theory of directed landing of dispersing individuals on susceptible trees during host selection does not hold true for I. grandicollis. The differences in the behaviour of the beetles subsequent to their landing, were detected only after they had bored into the inner bark. It appears that both resistant and susceptible trees are examined in the same way, while the decision to continue boring and establish themselves, or abandon boring and retreat, is made on an apparent message received from the bark.

The only apparent factor which prevented further attack on resistant trees was the resin produced by the damaged resin ducts and inner bark tissues.

Based on the results of this experiment the behaviour pattern associated with host selection in I. grandicollis seems to support Callaham's theory. Dethier (1953) described a similar behaviour pattern when explaining host selection in phytophagous insects, which involved orientation to food, biting response and continued feeding. It appears that in many polyphagous insects, specific chemical stimuli are not required to initiate biting, when plants are sampled randomly (Pfadt, 1949). Ips grandicollis is mostly monophagous in South Australia and selects its food plant after random landing on trees within its host range. (2)

The increased numbers of beetles caught on baited traps held on susceptible trees, after the initial two day boring period, is of interest. The increased attraction could be perhaps due to the enhanced production of the aggregation pheromone by the males that established themselves in such trees. If such a possibility is likely, it would also mean that the males that abandoned trees that were categorised as resistant did not produce the aggregation pheromone during the process of test boring. However, such a possibility until proven experimentally, remains a speculation.

In conclusion, the study shows that I. grandicollis adults upon emergence, randomly land on any host species, after which (2) the initial boring activity begins. Host discrimination occurs sometime during the test bore that extends upto the phloem-cambium interface. Perhaps, it may be that continued boring in resistant trees is regulated by the quantitative resin flow from the entry holes bored on trees as theorised by Callaham. The increased numbers of beetles caught in baited traps on susceptible trees may be explained if those beetles that remained began producing attractants, and the combination of natural and experimental ipsenol made the trees more attractive than they were with the ipsenol baits alone.

Such a proposition could be tested either by finding an absence of ipsenol in male beetles that leave resistant trees after initiating the test bores, and a presence of the pheromone in males that continue to bore into 'susceptible' trees or by demonstrating that additions of 'natural ipsenol' around baited traps do in fact enhance their attractiveness to dispersing beetles. Indeed both types of test are desirable to clarify the observed effects on 'susceptible' and 'resistant' trees.

SECTION 3

FACTORS CONTRIBUTING TO THE RESISTANCE OF P. RADIATA
TREES TO ATTACK BY I. GRANDICOLLIS

3. FACTORS CONTRIBUTING TO THE RESISTANCE OF P. RADIATA TREES TO ATTACK BY I. GRANDICOLLIS

3.1 Introduction

Many attempts have been made in the past to devise a framework within which the relationship between host and insect could be described in a way that would provide a definition of host resistance (Snelling, 1941; Painter, 1951, 1958; Beck, 1965; Stark, 1965). Hanover (1975), from his broad definition of the concept of host resistance indicated the complexity of this phenomenon. As any resistance response to insect attack should be manifested in the physiology of the host tree, all investigations pertaining to resistance have been directed towards a study of such mechanisms. Both environmental and genetical factors have been implicated as being responsible for development of such resistant responses (Gerhold *et al*, 1966; Hanover, 1975). Painter (1958) attempted to describe the resistance mechanisms in plants, under three categories a) preference and non-preference b) antibiosis and c) tolerance. Beck (1965) reviewed most of the early concepts on resistance in plants to insect attack. Hanover (1975) outlined four other host characteristics that are more specific and host orientated in describing tree-resistance mechanisms that have come into action due to an imbalance in the insect-tree relationship. These include 1) morphology and anatomy of the host 2) chemical repellents produced by plants 3) chemical attractants produced by plants and 4) nutritional status of the host.

Nevertheless, he further points out that the actual host resistance mechanism is most likely to be a combination or interaction of these conditions, either within the host itself or between host and insect. Specific mechanisms that lead to resistance of trees to bark beetle attack have been defined and reviewed by Beck (1965) and Stark (1965).

Of the many mechanisms that confer resistance to trees from bark beetle attack, exudation of resin resulting from mechanical damage caused by boring insects, has been considered a major factor. Hopkins (1902) recognised the trees' response to injury due to attack being manifested in the form of oleoresin, which in living trees is a major obstacle to successful establishment of galleries. Since then, such mechanisms have been pointed out by many others (Caird, 1935; Merker, 1960; Miller and Keen, 1960; Rudinsky, 1962; Reid, 1963 and Stark 1965). Investigators contributing to this field have attempted to explain the action of oleoresin against successful attack, on the basis of physical and chemical qualities of the resin. Studies by Smith (1961 a,b; 1963) have shown an association between the chemical characteristics of oleoresin and specific bark beetles; that led to host specificity. He further indicated that the toxic effect of resin is sublethal and that the beetle actually succumbs to the physical attributes of resin. Moreover, the characteristics of the tree that contribute to resistance are more obvious than the characteristics of the resin itself. These include a) the capacity of the tree to produce oleoresin expressed in terms of oleoresin exudation pressure (O.E.P),

oleoresin exudation rate (O.E.P.) (Anderson and Anderson 1968),
the ~~oleoresin flow rate (O.F.R.)~~ (Mason, 1969b, 1971) and
oleoresin consistency expressed in terms of viscosity and rate
of crystallization (Santamour, 1965). Stark (1965) has reviewed the
physical attributes of resin affecting bark beetle attack as well
as the various techniques employed in the measurement of O.E.P.,
together with the study of the environmental factors affecting
O.E.P. levels in trees.

It has long been recognised that coniferous trees whose
water balance is disturbed are specially susceptible to bark
beetle attack. Subsequently it was thought that moisture
deficiencies cause changes in moisture content and osmotic
pressure of the phloem sap. This led von Tubeuf (1917, 1933)
to erect the hypothesis that moisture stress causes susceptibility
in trees by reducing the resin production and exudation. This
idea was further advanced through the classical experiments of
Münch (1921) who showed that oleoresin exudation depends on the
turgor of cells lining the resin ducts or canals. Later work
confirmed this hypothesis (MacDougal, 1926; Ivanov, 1930;
Beal, 1930; Vité, 1961; Vité and Rudinsky, 1962). Since then
it has been a common practice among forest entomologists to
assess trees' resistance to attack in terms of O.E.P. Any
changes in water relations would also be reflected in the
xylem water potential. Moreover, xylem sap potential (non-
osmotic part of xylem water potential) and cell turgor are
in dynamic equilibrium. Hence any changes in sap tension
is reflected in cell turgor (Hodges and Lorio, 1971).

Therefore, sap potential (S.P.) has also been used as an indicator of cell turgor, as well as water disturbances in trees. Critical values of S.P. and O.E.P. relating to the ability of host trees to resist invasion by specific bark beetles, as well as threshold values of O.E.P. and S.P. below which bark beetle invasion is successful have been determined (Vité and Wood, 1961; Vité and Rudinsky, 1962; Wood, 1962; Wood and Stark, 1964). However, a reasonable doubt exists as to whether such data have a species-specific significance (Zwölfer, 1957) due to the wide diurnal and seasonal variations of O.E.P. obtainable from a single tree, as well as the wide variation of O.E.P. among trees from different sites. ✓

Nevertheless, the production of oleoresin still remains as an obvious characteristic of trees' resistance to bark beetle attack, and O.E.P. and S.P. have been considered the most accurate indicators of the physiological condition of host trees, as well as the decisive factor governing the ability of beetles to colonise hosts.

Although many observers have noted that the success of bark beetles in colonising trees is related to the ability of the host to produce resin, there have been only a few definitive studies on the interrelationship between a specific bark beetle, its host species and O.E.P. (Wood, 1972; Vité and Wood, 1961, Wood, 1962; Vité and Rudinsky, 1962).

An experiment was designed to study the interaction between Ips grandicollis and its host Pinus radiata, in relation to O.E.P.

and S.P. levels of the host that may lead to successful colonisation of the tree. In the first phase of the study P. radiata trees were subjected to certain treatments that are known to predispose Pinus trees to bark beetle attack, following which their vigour has been assessed by measuring their O.E.P. and S.P. levels. In the second phase of the experiment, susceptibility of these trees to attack by I. grandicollis has been tested. Susceptibility of the trees was assessed (under a rather artificial situation) by the ability of caged beetles to attack trees subjected to the various treatments.

3.2 Methods and materials

The investigation was carried out in a 10-year-old P. radiata plantation at Mortlock Experimental Station, 150 km north of Adelaide, South Australia. This plantation had no natural infestation of I. grandicollis. Fifty trees of dbh (18.8 to 25.9 cm) were randomly selected within this 1 ha plantation. Simultaneous measurements of O.E.P. and S.P. levels of these 50 trees were made 3 times a day (before sun rise, at mid day and after sunset) to account for the diurnal fluctuations of these levels. Based on their existing mean O.E.P. and S.P. measures, the trees were classed into 2 risk-rate categories. Equal numbers of trees, selected randomly from both groups, were subjected to treatments, that are known to predispose pine trees to attack by bark beetles. Following such treatments, beetles were caged on the boles of test trees. Measurement

of O.E.P. and S.P. levels were continued throughout the 3-week period during which beetles were caged on the trees.

3.3 Experimental techniques

O.E.P. levels in P. radiata trees were measured using a simple technique described by Wolfe and Maple (1965) and Mason (1969b). This method uses capillary tubes with one end blocked and a plug of mercury located within the bore of the capillary. The open end of the capillary tube is fitted tightly into holes drilled into the sapwood of the trees.

Severing of resin canals as a result of this operation, causes exudation of resin, which finds its way into the bore of the capillary tube. Tubes were inserted early in the afternoon and left for 4hr until the resin flow reached equilibrium.

Variation in O.E.P. is indicated by changes in air volume inside the capillary tube and is obtained by measuring the distance between the mercury plug and the inner edge of the sealed end. This measurement is compared with the measurement taken previously just after the capillary was located in the tree. Measurements were corrected for any variation in temperature. Four capillary tubes inserted into each tree at b. h. in the 4 cardinal directions, were used to eliminate any directional effects at any one time on the O.E.P. levels.

The pressure chamber technique of Scholander et al. (1965) was used to estimate the S.P., which estimates the non-osmotic component of xylem pressure potential of trees. For the estimation of S.P., leaf-needles were used as they are a

reliable indicator of S.P. and more easily sampled than twigs. Two needles from the new growth of the first living branch of each tree on the northerly aspect were sampled at each time. The needle to be used for the S.P. measurements was cut off the twig with a razor blade. It was inserted with its free end protruding out, through a rubber compression gland in the lid of the pressure bomb. As the pressure in the bomb is increased, the sap suddenly moves to the surface of the cut end, which is viewed through a lens. The pressure at which the liquid wets the surface equals that pressure which existed in the needle, before it was severed from the twig.

Soil moisture measurement at 7 sites adjacent to the trees that were randomly selected, was determined using an electron probe. Soil moisture measurements were taken at 30 cm, 60 cm and 1 m depths. The probe was calibrated using the gravimetric measure of moisture content of soil cores, excavated from the respective depths.

3.4 Data analysis

Data for each of the two main parameters, O.E.P. and S.P., for the 49 randomly selected trees were analysed in a 3-way analysis of variance to determine :

- a) between tree variation of O.E.P. and S.P.
- b) between time variation of O.E.P. and S.P.
- c) (time x tree) interaction variation of S.P. and O.E.P.

Results of the analysis given in Appendix 3.1 and 3.2 indicate a significant difference in S.P. and O.E.P. measures, both between trees as well as between times at $P = 0.01$. The interaction between times and trees was not significant for either of the parameters. The sampled trees had a mean O.E.P. of 3.1 bars (range 1.3 to 5.4) and a mean S.P. of 23.3 bars (range 17.0 to 27.0). Of these two measures, O.E.P. was used as a basis for categorising the sampled trees. Based on their mean O.E.P. level, trees with O.E. P. levels ranging from 1.0 to 2.5 bars were put into one category and those trees with O.E.P. levels over 3.5 bars into the next category. The test trees in the former category were expected to have a high attack risk, while trees in the latter category a low attack risk.

3.5 Experimental treatments

An equal number of trees were selected from each of these two risk-rate categories and were subjected to the following treatments :

- 1) Girdled at their base, ≈ 20 cm from the ground (B.G.).
- 2) Girdled below the lowest living branch or crown girdled (C.G.).
- 3) Left intact (not girdled). (L.I.)
- 4) Felled and left otherwise intact. (F)
- 5) Felled with all the branches lopped leaving only the boles.

O.E.P. levels and S.P. levels of the test trees were determined before subjecting them to the above treatments, as well as at the end of the 3-week period following caging of beetles on them.

3.6 Caging the beetles

Beetles emerging from field-infested logs were caged at 4 different heights on the bole of all test trees. Each cage (22) shown in Fig 3.1.A was made from a plastic petri dishes (3 cm diameter). A central hole of 0.5 cm diameter was cut on the top surface for the introduction of beetles. A piece of foam plastic was glued round the rim of the petri dishes to ensure a close fit between the tree and the cage. The cages were held on to the tree with wire. Ten beetles were released into each (23) cage, which was left on the tree for a period of 3 weeks. Dead beetles were replaced every week. Beetle attack was evaluated at the end of the 3-week period by debarking and examining the area under cages.

3.7 Evaluating beetle attack

The initial biting response was identified from the dark pink frass collected in the cages. On removal of cages the following observations were recorded for each cage site on the tree.

- a) Number of entry holes
- b) Depth of penetration into bark
- c) Number of nuptial chambers
- d) Nature and number of galleries constructed in inner bark

Fig 3.1 (A) shows the cage that was used to confine

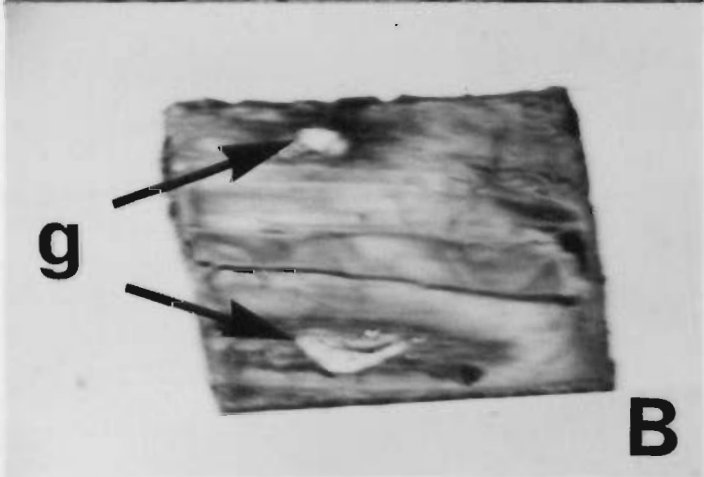
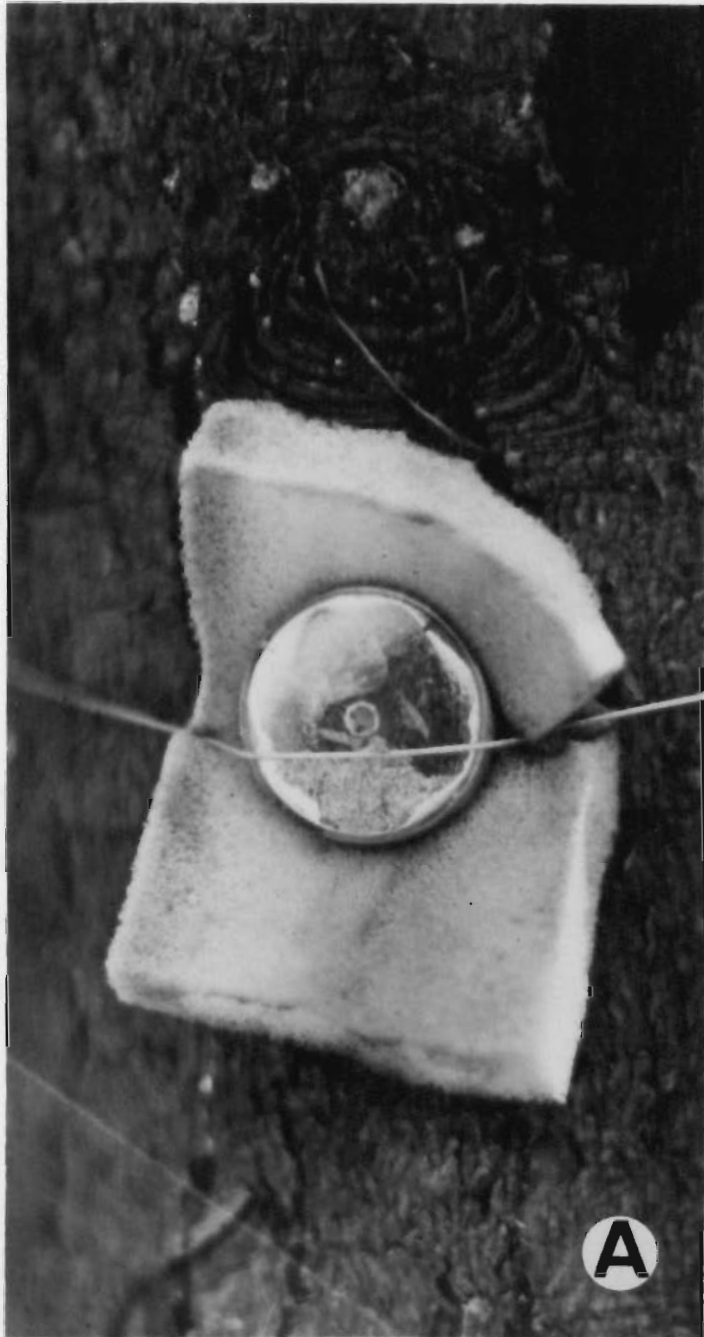
I. grandicollis adults to the bole of

P. radiata trees.

(B) Shows the area under bark at one of the cage

sites in an intact P. radiata tree.

Note the short, broad galleries covered in
resin.



The nature of the attack was evaluated using an attack score system that was based on the depth of penetration into the bark as shown in Fig 3.2 and given in Table 3.1 The scores on the nature of attack at each of the cage sites on the boles of the trees treated differently are given in Appendix 3.3. Treatments (other than where successful establishment occurred, i.e. in felled trees) were compared with one another, using the Mann-Whitney ranked non-parametric test (Siegel, 1956).

3.8 Results

3.8.1 Soil moisture level

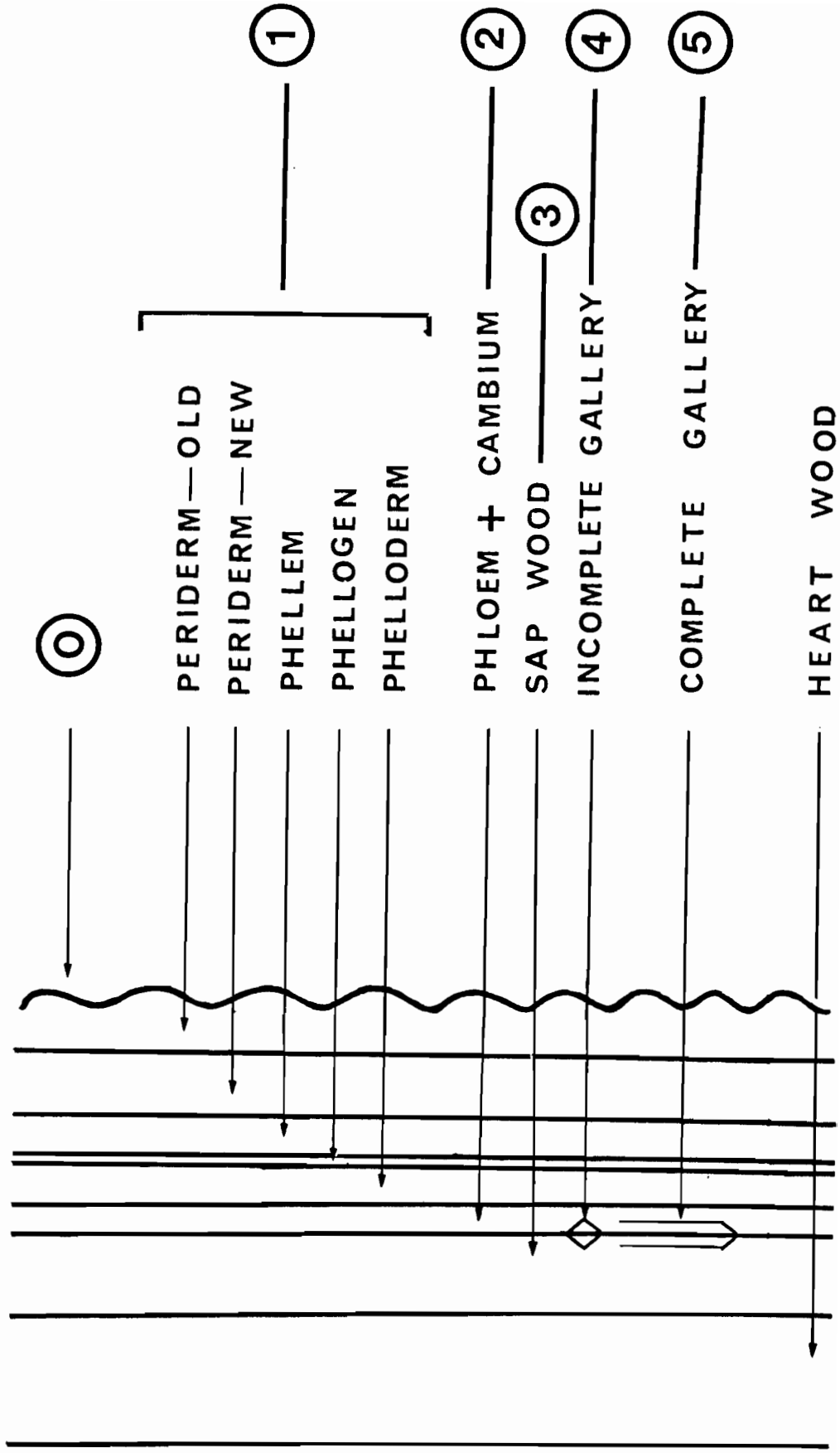
The soil moisture levels at the 7 sites adjacent to the test trees did not differ significantly as shown in Appendix 3.4. However, the moisture content differed significantly with depth. The mean moisture content at 30 cm, 60 cm and 1 m depths were 206 ± 14 ; 154 ± 7 and 150 ± 15 per cent o.d.w. respectively.

3.8.2 O.E.P. and S.P. Levels

The effect of various treatments on the selected trees in relation to their O.E.P., S.P. and on the number of entrance galleries made by I. grandicollis beetles, is given in Appendix 3.5. No significant difference in any of the 3 parameters were observed for any of the treatments, other than in trees that were felled.

Fig 3.2 Diagrammatic L.S. of P. radiata stem showing the depths of penetration into the bark by boring I. grandicollis adults.

- 0 -No boring
- 1 -Boring through outer bark
- 2 -Boring into inner bark
- 3 -Boring through inner bark upto the surface
of sapwood
- 4 -Construction of incomplete galleries which
were later abandoned
- 5 -Construction of galleries leading to
establishment.



1

2

3

4

5

Table 3.1

Attack scoring system

Score	Nature of attack
0	No boring
1	Boring only in corky bark
2	Boring upto phloem
3	Penetration of phloem followed by boring upto sapwood
4	Construction of short, broad, irregular galleries
5	Construction of nuptial chambers and egg galleries.

3.8.3 Beetle attack

Similarly, the number of attacks on trees did not differ significantly with treatment or on trees left standing (Appendix 3.6). However, in felled trees a mean number of 27 ± 1.0 attacks per tree was recorded as against a mean number of 5 ± 0.2 attacks per tree on trees subjected to the other 3 treatments.

The nature of attack evaluated using the scoring system (Table 3.1) did not indicate any significant difference between the 3 treatments on analysis (Table 3.2). The values indicate that for each comparison, the hypothesis : that there was no difference between treatments in relation to the nature of attack should be accepted. Attacks in all trees had penetrated upto the phloem-cambium layer, after which the attacks were abandoned by the beetles. A few beetles were found dead at the phloem-sapwood interface, while those that were outside the galleries were covered in resin. Though short, broad irregular, vertical galleries and incomplete nuptial chambers as shown in Fig 3.1.B, were present in both treated and intact standing trees from both categories, egg galleries were observed only in felled trees. However, even in the felled trees, successful attack leading to establishment did not occur until 2 weeks after felling.

Table 3.2

Comparison of attack scores from each of the cage sites on treated trees. (Analysis based on Mann-Whitney ranked non-parametric test (Siegel, 1956))

Treatment comparison	Value of U	Probability
BG vs CG	169.0	0.18 (a)
CG vs LI	364.57	0.50 (a)
BG vs LI	291.6	0.34 (a)

Probabilities followed by the same letters not significant. Hence the hypothesis that there is no difference between treatments is accepted.

3.9 Discussion and conclusion

The initial survey of the 49 trees indicated a significant variability in their O.E.P. and S.P. levels, despite the fact that the trees sampled came from a stand of trees of the same age, and where the soil moisture levels at different sites over the stand did not differ significantly. (19)

From the results of caging beetles on test trees, it can be concluded that despite the effect of some of the treatments known to predispose Pinus trees to successful attack by bark beetles, caged beetles failed to establish themselves on P. radiata trees subjected to such treatments.

However, the hypothesis that I. grandicollis discriminates between resistant and susceptible trees after the initial boring activity, seems to be supported from the observations of this experiment in that the beetles, after boring upto the phloem-cambium region of the bark of standing trees, either had left the tree or were seen drowned in the resin. The presence of such attacks on trees with O.E.P. > 3.5 bars as well as on trees with lesser O.E.P. values, suggests that all trees are equally susceptible, at least to this preliminary attack. Nevertheless, the nature of the preliminary attack itself differed, even among similarly treated trees as well as on the same tree. In the same tree, while some beetles had bored only upto the phloem-cambium interface, after which they had abandoned the entrance gallery, others had begun to initiate vertical galleries, in which they were found drowned in the resin on debarking.

Though the beetles failed to establish in treated standing trees, indicating a marked difference between them and felled trees, the delay in establishment in felled trees indicates that factors other than the absence of O.E.P. itself are involved in the rejection of a tree for establishment. However, under the experimental conditions where the beetles were induced to attack selected trees, the natural interaction that occurs between the insect and the host is lost, due to the absence of free choice in host selection. Yet the results of this experiment indicate that even 'healthy' trees are susceptible to initial boring, even though such attacks were unsuccessful, and did not lead to successful establishment.

Previously when attacks were induced on living P. radiata trees from natural Ips grandicollis populations using the aggregation pheromone, ipsenol, a large number of so-called 'test bores' which were later abandoned, had been observed. (see Section 2).

It appears that the process of host selection in I. grandicollis involves random landing followed by initial boring, and the success of the attack is governed by the subsequent reaction of the host to such activity. Attempts to establish in living trees as indicated by initiation of vertical galleries observed in this experiment may be due to a delay or failure in the resinosis response in such trees. (2)

In the absence of any measurable O.E.P., the ability of phloem tissues to produce secondary resin may render a tree

unsuitable for establishment. Anderson and Anderson (1968), from a study of lightning struck pine, showed a definite relationship between oleoresin exudation rate (O.E.R.) and success of Ips attack. In Ips grandicollis, no attacks succeeded or establishment occurred in trees where O.E.R. exceeded 0.1 ml/hr from a standard wound.

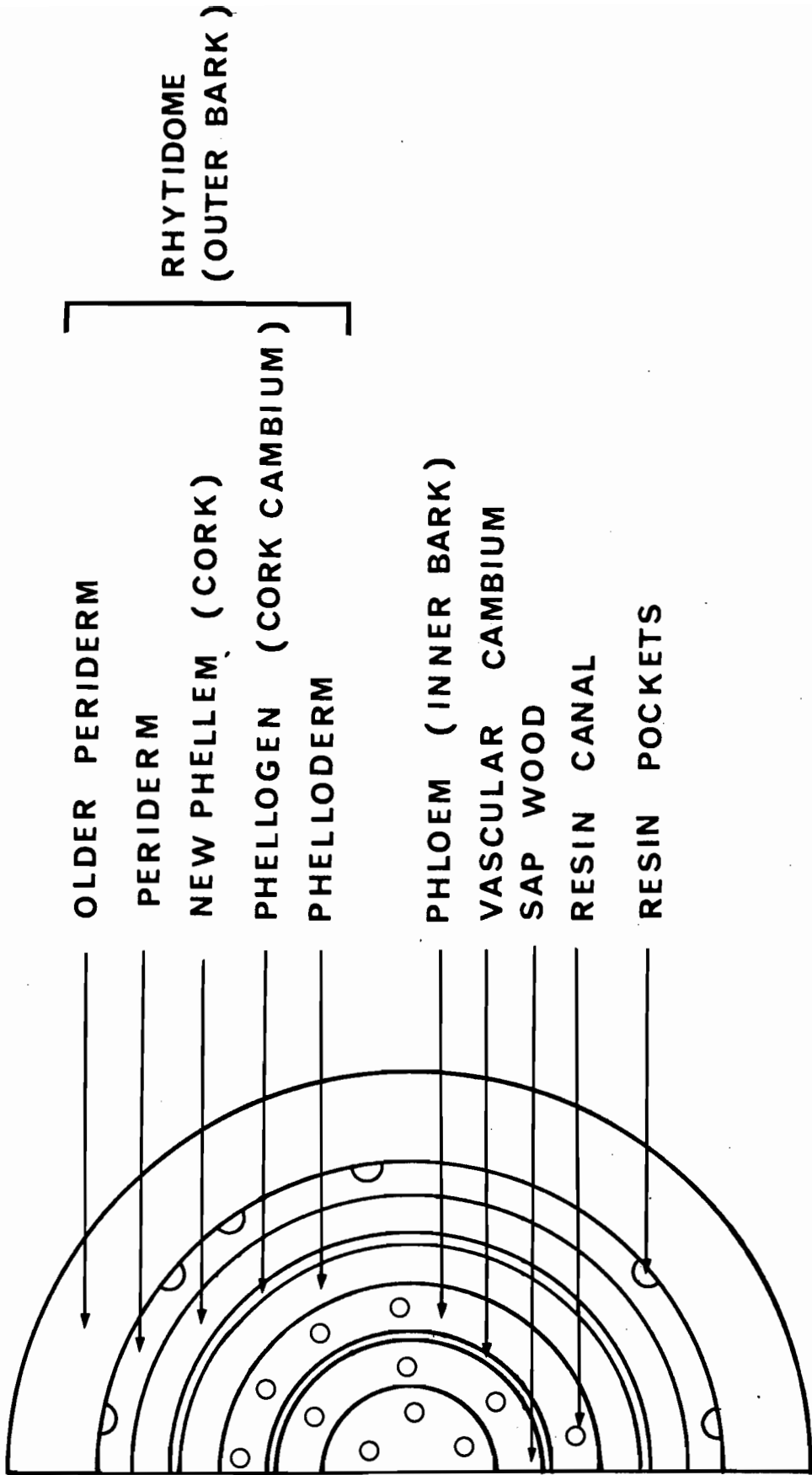
Nevertheless, the results of this experiment indicate that establishment in felled trees does not occur in the presence of any exuding resin, or as long as the phloem tissues had the ability to produce secondary resin on injury.

Moreover, all the techniques presently used in measuring O.E.P. in trees involve drilling as far as sapwood or inner xylem and the oleoresin from the resin ducts in the sapwood thus severed finds its way into the capillary tube. Hence, such a technique will only measure the O.E.P. of resin from the resin-ducts present in the xylem region.

Patel (1975) from a detailed macroscopic and microscopic study of the bark anatomy of conifers, describes the presence of horizontal resin canals and the absence of any axial resin canals in the inner bark (\equiv phloem) of P. radiata. The xylem of P. radiata, on the other hand, bears both axial and horizontal resin canals. Moreover, the bark of P. radiata bears numerous blister-like cavities in which resin is contained (authors personal observation).

From the above observations on the anatomy of the outer and inner bark as shown in Fig 3.3, it appears that injury to either

Fig 3.3. Diagrammatic T.S. of P. radiata stem(after Patel, 1975) showing the distribution of resin barriers.



the resin reservoirs in the outer bark, or to the horizontal resin canals in the inner bark by beetles boring entrance galleries, would lead to unsuccessful attack on living trees. However, no study has been carried out so far to determine the distribution and abundance of such resin bearing structures in a given area of bark surface. If such data were available, the probability of a boring beetle encountering such resin barriers could be assessed. From casual observations it appears that failure to encounter these barriers would permit the beetle to continue boring, until it was prevented from doing so by the secondary resin produced by injured phloem cells. Any delay in this response may lead to initiation of vertical galleries as observed in this experiment.

Thus it is most likely that a boring beetle would encounter the resin ducts in the outer sapwood, whose pressure is indicated by the usual O.E.P. pressure measurement last of all, and only in the absence of all other previously mentioned resistance mechanisms. Hence a measure of O.E.P. alone will not give an accurate account of the resistance mechanisms encountered by a boring beetle, specially because, as this study indicates, the decision to abandon or continue boring is made before reaching the sapwood of the host. Therefore, it is more likely that secondary resin production by phloem cells is a major decisive factor governing success of beetle attack. Also the relationship between the resin ducts in the xylem region and those in the phloem tissue is not known. Hence to what extent the xylem O.E.P. reflects

the O.E.P. in the resin ducts of the phloem cannot be assessed.

All these observations finally relate to the effect that O.E.P. by itself is not a suitable indicator of P. radiata trees' susceptibility to attack by I. grandicollis. But specially in its absence, the ability of phloem cells to produce secondary resin, is a better criterion for assessing susceptibility of trees to initial I. grandicollis attack as well as to successful establishment.

At high beetle population densities as present during endemic as well as outbreak situations, a different type of interaction may come into operation. But under low population levels, I. grandicollis is able to establish only in P. radiata trees of very low vigour, where the O.E.P. is zero and where the phloem cells have lost their ability to produce secondary resin. It is apparent from this study that the phloem in felled trees retains this ability for about 10 days from felling.

SECTION 4

MATING BEHAVIOUR OF I. GRANDICOLLIS :
EVIDENCE FOR PRE-EMERGENCE MATING AMONG MATURE PROGENY

4. MATING BEHAVIOUR OF I. GRANDICOLLIS : EVIDENCE FOR PRE- EMERGENCE MATING AMONG MATURE PROGENY

4.1. Introduction

One of the mechanisms involved in the aggregation of bark beetles is the response to insect-produced attractants. In recent years much research on these pheromone systems have led not only to the identification, isolation and synthesis of the substances concerned, but also to the conditions of their production.

Ipsenol (2-methyl-6-methylene-7-octen-4-ol) has been isolated from the hind-gut volatiles of the male adults of I. grandicollis and identified as the pheromone responsible for the aggregation of the sexes (Vité and Renwick, 1971). This pheromone is known to direct the dispersing individuals to the host selected by the males that initiate attack (Vité et al, 1976; Hedden et al, 1976). Hughes (1974) demonstrated that myrcene, (2-methyl-6-methylene-7-octene), a host monoterpene can serve as a precursor of the pheromone ipsenol. He further showed that I. grandicollis requires feeding for the biosynthesis of myrcene into ipsenol, unlike I. avulsus or I. paraconfusus that produce the pheromone without prior feeding but on mere exposure to myrcene vapour.

The importance of feeding in the biosynthesis of ipsenol by I. grandicollis indicates some form of control over its production. The pheromone synthesised is excreted along with the faecal pellets, which together with the chewed-up ^{inner} bark constitute the frass. ✓

Werner (1972b) demonstrated that for I. grandicollis, the attractiveness of this male-produced frass decreased with mating, where frass from virgin males was found to be far more attractive compared with frass produced by a mated pair. However, the response of newly emerged females differed in their response to whole frass vs frass extract, from mated and unmated males. While no significant difference was observed in the response to frass from virgin males and mated males, a significantly greater response was observed with the respective frass extracts. However, the response of males to the two kinds of frass as well as to frass extracts differed significantly. These studies indicate the importance of mating on the attractiveness of frass produced by males of certain Scolytid species.

Bark beetles in the genus Ips DeGeer aggregate on trees, in response to the male-produced attractant or the aggregation pheromone. Following landing of the responding beetles on the trees selected by males, females have been observed in nuptial chambers that are initiated and constructed by the males. Stridulation of females has been found to precede the entry of these females into nuptial chambers, constructed by the males (Wilkinson et al 1967). After they have mated, females typically proceed to construct egg galleries that radiate from the nuptial chamber. Such a behaviour pattern has been recorded for several species of bark beetles in the genus Ips (Barr, 1969 for I. confusus; Schmitz, 1972 for I. pini; Wilkinson et al 1967 for I. calligraphus; Gouger et al 1975 for I. avulsus; Bungey, 1966 and Morgan, 1967

for I. grandicollis). This behaviour pattern led to the (24)
 "assumption" that all adults emerging for the first time from the sites of
 development were unmated. Any females in the dispersing
 population that were found to have mated, were therefore considered
 as parent adults that had already established progeny were
 moving to new sites to produce further.

However, Mc Cambridge (1969 a, b) suggested that Ips ?
 and related bark beetles mate early following maturation. He
 found that 15 per cent of the females of Dendroctonus ponderosae not Ips
 Hopkins that emerged from infested logs contained spermatozoa,
 whereas in laboratory rearings less than 2 per cent of the
 emerging females were fertilised. He concluded that mating
 had probably occurred in the collecting jar after emergence.
 Examination of freshly-emerged females of Dendroctonus monticolae Not Ips
 Hopkins, showed that less than 1 per cent had mated before they emerged
 and that the mated females had been the last to emerge (Reid, 1958).
 Wilkinson (1964) reported that solitary I. grandicollis females that (25)
 responded in the field to artificially infested bolts or logs were
 capable of producing progeny in much the same way as the females
 closely associated with males in their nuptial chambers. Similar
 findings made by All and Anderson (1972) indicated that, of the
 females that emerged from brood logs 62 per cent were capable of
 producing progeny. Both these authors assumed such females to be (26)
parent adults that had retained sperms from a previous mating.
 Any possibility of parthenogenetic reproduction in I. grandicollis
 has been ruled out on the evidence of All and Anderson (1972).

These findings prompted investigations into the earliest period in a beetle's life that mating could occur. Such information in the light of the observed variation in the attractiveness of frass with mating (Werner, 1972b) would be of significance to future studies on insect-produced attractants and specifically to their production and the response to them. Hence an investigation into the mating behaviour of I. grandicollis seemed appropriate. In view of the observations and assumptions (26) made by Wilkinson (1964) and, All and Anderson (1972), it was necessary to determine whether parent females contributed to the incidence of mating observed among the emergent females. Hence the initial study involved the examination of dispersing I. grandicollis females as well as those emerging in the laboratory from field-infested logs, to determine the proportion of mated females in these populations. Secondly, an effort was made to differentiate between the 'parent adults' and 'progeny adults' in order to determine any incidence of mating among the progeny. The possibility of individuals mating on emergence, outside the galleries constructed under bark was also considered. In the course of the work, related studies were conducted to determine the influence of time spent under bark by mature beetles on the incidence of mating therein, and also to consider any possibility of mating among siblings.

4.2. Materials, methods and results

4.2.1. Survey of the dispersing population

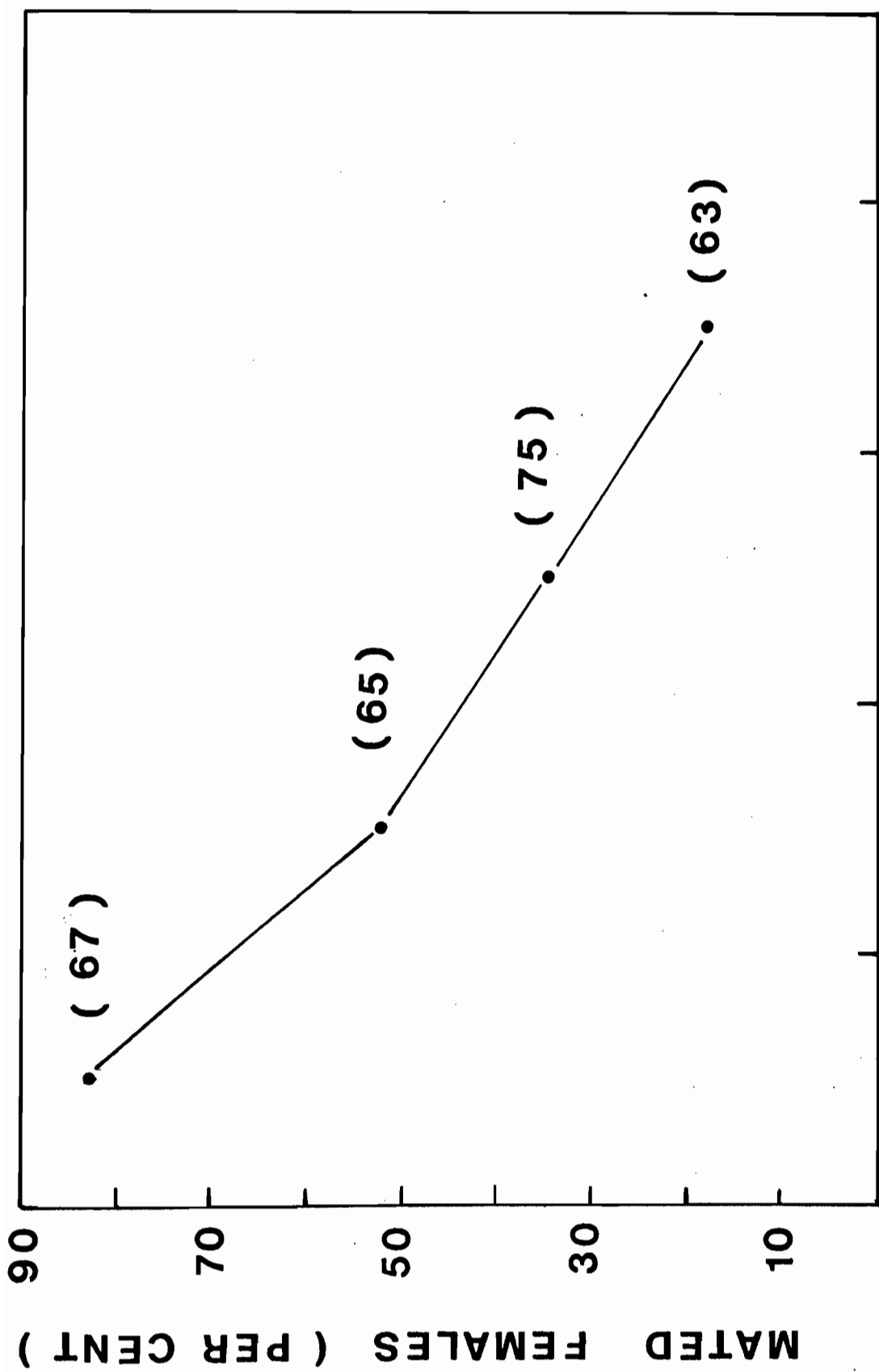
This survey was carried out in an infested P. radiata plantation at Mt Crawford 60 km N.E. of Adelaide, South Australia. The flying beetle population was sampled throughout the emergence period (mid November to late March) of 1976 to 1977. Dispersing beetles were attracted to Tangle-foot[®] coated traps using baits containing the aggregation pheromone, ipsenol. Ten traps were deployed over an area of 1 ha and the adults removed from them every 2 days for 8 days.

The individuals collected were placed in 90 per cent ethanol to remove the Tangle-foot[®]. They were then sexed ~~according to the morphological characters of the frons~~ ^{based on the presence of stimulation organ on the head of females?} (Wilkinson, 1962). A (27) random sample of the females was dissected (under x 32 magnification) and their spermathecae examined. The presence of spermatozoa in their spermathecae was used as an index of mating. The results (Fig. 4.1) indicate that the proportion mated was high (87 per cent) early in November and thereafter declined (16 per cent) towards the end of the emergence period.

4.2.2. Examination of females immediately after their emergence

Infested logs were collected from the field about the time adults were due to emerge. The logs were caged in the laboratory and maintained at 25°C. The cages had a metal frame with sides covered with ~~transparent~~ ^{translucent?} muslin cloth. Beetles on emergence tended to settle on the sides of the cages, being attracted to light. In order to obtain newly emerged beetles, cages were

Fig 4.1 Percentage mated females of Ips grandicollis
in the dispersing population caught on
pheromone-baited traps. The number dissected is
denoted within parenthesis.



NOV DEC JAN FEB MAR

— SPRING — SUMMER — AUTUMN —

cleared of beetles each morning and those settling on the sides of the cage during the day were caught. As peak emergence occurred in the afternoon, those emerging during this time of the day were selected for the experiment. A proportion of the females caught were examined for the presence of spermatozoa in their spermathecae. The population that was sampled in this experiment consisted of emergent adults from 5 cages, with each cage bearing 8 to 10 logs (35 cm x 12 cm). Of the females that emerged in November, 89 per cent were found to be mated, compared with 40 per cent mated in the March emergents (Table 4.1.). Closer observations of the emerged beetles did not indicate any mating behaviour, either on the surface of the log or on the walls of the cage.

4.2.3. Proportion of mated females among emergent progeny

A simple technique was developed to mark the 'parent adults' before releasing them into logs to produce progeny. Using a pin head, a spot of coloured varnish was applied to the elytral declivity of both sexes. This method enabled clear differentiation of the adult progeny resulting from the parent adults. Moreover, there is usually a lag between parent adult emergence and progeny adult emergence, the former always emerging earlier. Into each cage containing 4 logs (ca. 35 cm x ca. 10 cm), a total of ca. 60 marked females and ca. 20 marked males were released to produce progeny. A total of 6 cages ^{was} were used. Three of these cages were maintained at 25°C and the other 3 at 30°C.

The unmarked progeny adults were collected as they emerged and a random sample was assessed for any incidence of mating, as in previous experiments. About 8 per cent of the emergent progeny were found to be mated (Table 4.2) at each temperature.

4.2.4. Possibility of mating taking place after emergence and outside galleries

The hypothesis that adult beetles could mate after emergence prior to dispersal, and outside galleries, needed to be tested to confirm the results of the previous experiment. Hence the bark sandwich technique of Reid (1962) was adopted. This technique enables rearing of beetles through a generation. Progeny produced in bark sandwiches from mated females, was removed at the callow adult stage. The individuals were sexed, and the sexes were held separately in petri dishes upon chopped up moist bark, until maturity. Callow adults following eclosion undergo a series of colour changes from pale brown through shades of reddish-brown, to attain their final chocolate-brown colour, over a period of 2 to 3 weeks at 30°C. Dissection of both males and females, as they proceed through this colour change, indicated that individuals are not sexually mature until they appear reddish-brown in colour. This has been inferred from the presence of motile sperms in the testes of the male, as well as in the spermathecae of the female. Active mature individuals of reddish-brown colour were caged in petri dishes lined with moist filter paper in the ratio of 1 ♂ : 3 ♀ (the average sex-ratio

Vita &
Rudinsky
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Acrobactonus

Table 4.1

PROPORTION OF MATED I. GRANDICOLLIS FEMALES AMONG EMERGENTS FROM
FIELD-COLLECTED LOGS

Time of collection of logs from the field	No. emerged (♂ and ♀)	No. emerged (♀ only)	No. dissected (chosen at random)	% mated
	$\bar{s} \pm \text{S.E.}$	$\bar{x} \pm \text{S.E.}$		
November (early)	401 ± 17	187 ± 10	60	89
January (early)	388 ± 14	104 ± 8	41	58
February (late)	480 ± 20	214 ± 15	68	49
March (late)	420 ± 8	198 ± 7	49	(40)

Table 4.2

PROPORTION OF MATED FEMALES AMONG LABORATORY-REARED PROGENY POPULATIONS
(DATA FROM 3 REPLICATES OF MARKED PARENTAL BEETLES AT EACH OF 2
TEMPERATURES)

Generation* time in days	Rearing tempera- ture	emerged/ replicate (♂ + ♀)	No. females	No. dissected	% mated
	°C	$\bar{x} \pm \text{S.E.}$	$\bar{x} \pm \text{S.E.}$		
44	25	165 ± 7	94 ± 9	48	8
38	30	202 ± 9	117 ± 5	59	9

* Time from release of parents to commencement of emergence of progeny

in natural infestations) and left for up to 48 h at 25°C. This process was repeated thrice, and at the end of the exposure period, females were assessed as in previous experiments. A total of 30 virgin females were used in the experiment. No spermatozoa were found in any of these females, indicating that mating ^{did} ~~does~~ not occur under the experimental conditions.

4.2.5 Incidence of mating among progeny adults

On rejecting the hypothesis that adult beetles do mate outside their galleries, a second hypothesis was erected to test whether adult progeny could mate prior to emergence while still under bark. An experiment was set up where four mated females were introduced into a single bark sandwich through separate entrance holes, and were allowed to produce progeny. The resulting progeny from different females, as well as from the same female were seen to mix and come in close contact with each other within the bark sandwich. The experiment was replicated 10 times and the bark sandwiches were maintained at 30°C, throughout the study. When the progeny attained the reddish colour (approximately after 4 weeks) they were removed from the bark sandwiches. The progeny adults were sexed and a random sample of the females dissected and examined as previously described. About 58% of these females were found to be mated (Table 4.3).

Table 4.3

Proportion of mated females among progeny reared within bark sandwiches (data from 10 replicates, examined 3 weeks following eclosion of adults at 30°C).

No. of females produced per replicate $\bar{x} \pm \text{S.E.}$	No. dissected per replicate $\bar{x} \pm \text{S.E.}$	Percentage mated $\bar{x} \pm \text{S.E.}$
24.9 \pm 0.9	15 \pm 0.7	58 \pm 1.8

4.2.6. Influence of time spent under bark on the incidence of mating

Based on the results of the above experiment, the hypothesis that mating could occur under bark prior to emergence was accepted. On further consideration of this hypothesis, it seemed warranted to determine whether the variable time spent under bark by mature adults had any effect on the incidence of mating. To test this hypothesis the previous experiment was repeated except that the adults produced within bark sandwiches were allowed to remain for further periods of one, two, three and four weeks following their maturation. Five replications were used for each time. At the end of each period, the females produced therein were examined as previously. As indicated in Fig 4.2. the proportion of females that were mated increased from 8 per cent to 74 per cent with increased time within the bark sandwiches.

4.2.7. Possibility of sibling mating

Judging from the evidence that progeny adults mate prior to emergence, it was speculated that progeny from the same batch (produced by a single mated female) could mate and produce viable progeny. Again the bark sandwich technique was adopted, except that a single mated female was introduced into each bark sandwich. Ten similarly constructed bark sandwiches were maintained at 30°C until the progeny adults attained the reddish brown colour. On examination of the randomly selected females, 54 per cent were found to be mated (Table 4.4). The remaining females were put back into fresh sandwiches to determine the viability of their offspring. Of those females that oviposited (49.8 per cent), almost all produced viable adults (Table 4.5).

Fig 4.2 Percentage of mated females of I. grandicollis
among progeny adults reared and held in bark
sandwiches for varying periods.

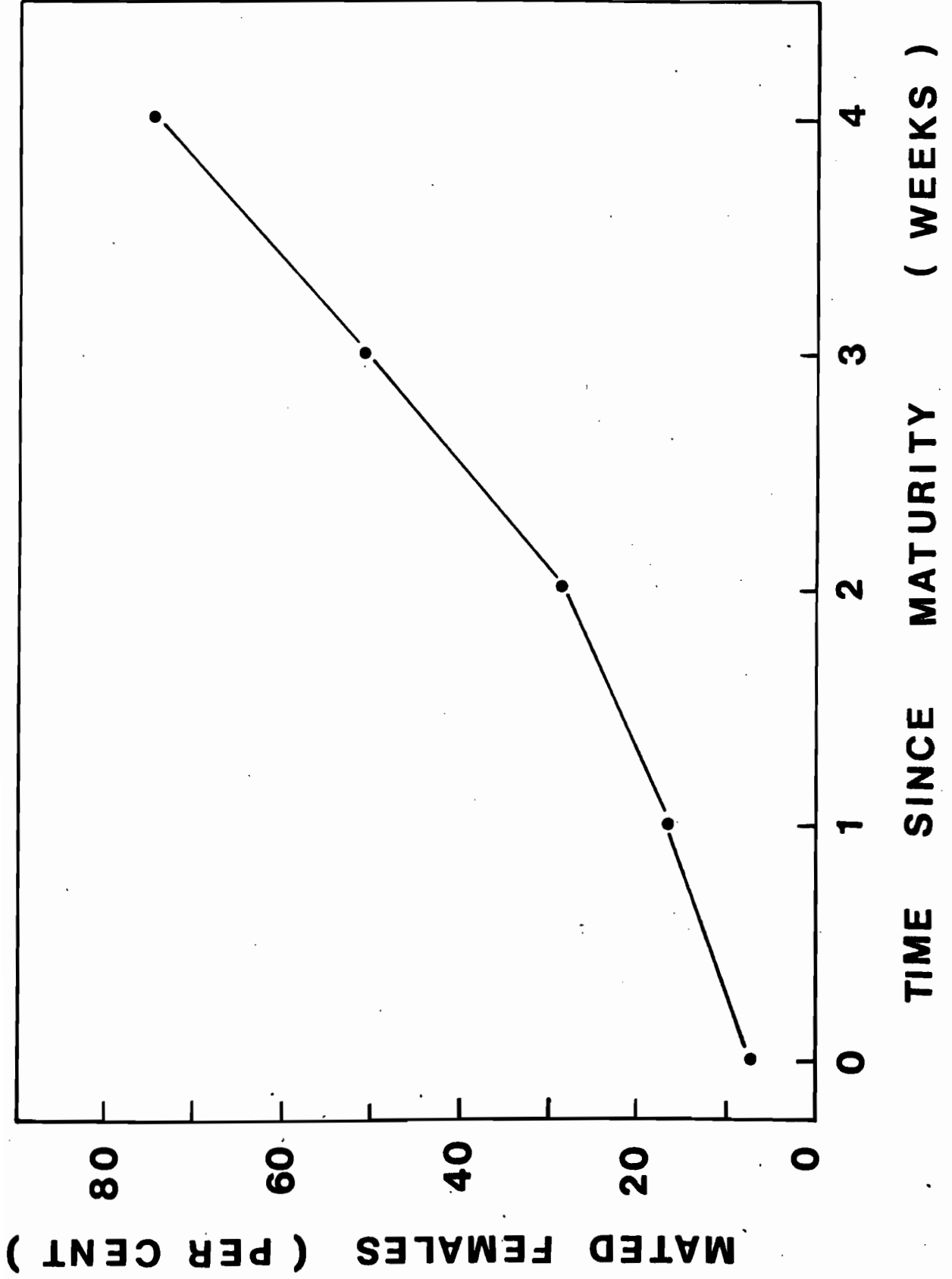


Table 4.4

Proportion of mated females among siblings reared within bark sandwiches (data from 10 replicates, examined 3 weeks following eclosion of the adult at 30°C).

Total No. females produced per replicate $\bar{x} \pm \text{S.E.}$	No. dissected per replicate $\bar{x} \pm \text{S.E.}$	Percentage mated $\bar{x} \pm \text{S.E.}$
10.6 \pm 0.7	5.0 \pm 0.3	54 \pm 2.4

Table 4.5

VIABILITY OF EGGS RESULTING FROM SIBLING MATINGS (DATA FROM 5 REPLICATES EXAMINED 2 WEEKS FOLLOWING ECLOSION OF THE ADULT AT 30°C)

% ovipositing females per replicate $\bar{x} \pm \text{S.E.}$	No. eggs per female per replicate $\bar{x} \pm \text{S.E.}$	% Reaching adulthood per replicate $\bar{x} \pm \text{S.E.}$
49.8 \pm 1.2	30.4 \pm 1.8	98.2 \pm 2.4

4.2.8. Attractiveness of mated versus virgin females to males in nuptial chambers

Evidence so far collected clearly showed that ^{some} mating takes place prior to emergence in I. grandicollis. A further experiment was designed to test the hypothesis that a mated female would be less likely to be accepted into a nuptial chamber by a male than a virgin female. For this purpose males were allowed to construct nuptial chambers within bark sandwiches by releasing them singly through holes drilled on the top plate of the sandwich.

On completion of construction of the nuptial chambers, both virgin as well as mated females were released singly at the entry point to the nuptial chamber.

Virgin females for the experiment were obtained from cultures of progeny females that were segregated at the callow adult stage and reared on chopped up bark until they were sexually mature. Mated females were obtained from field-infested logs. On debarking a log into which male and female adults were released to reproduce, the females that were ovipositing or found in association with a male, were declared mated and used in the experiment. Twenty trials using 20 males in nuptial chambers were used to test the acceptance of both virgin and mated females. Both mated and virgin females were equally accepted by resident males of the nuptial chambers. Moreover, both kinds of females were accepted by the same male.

4.3. Discussion and conclusion

The data presented indicate that adults of I. grandicollis mate in galleries under bark prior to their first emergences from the host in which they developed. The likelihood that mature adults may mate on the outside of logs or on the walls of containers as speculated by Wilkinson (1964) and All and Anderson (1972) has been clearly ruled out for I. grandicollis. The proportion of the insects that mate prior to first emergence is largely determined by the time spent under bark following maturity of the beetles. (28)

The high proportion of mated females observed in the dispersing population as well as among the individuals emerging from infested logs, was in agreement with the findings of Wilkinson (1964), All and Anderson (1972). The decreasing trend observed in the proportion of mated females in the dispersing population from early November (spring) to late March (autumn) could be explained in terms of their biology. I. grandicollis produces about 3 to 5 generations a year in South Australia. The generation that develops through winter (June to September) takes up to 6 months from egg to adult emergence, as larvae and adults over-winter. ✓

However, the summer (December to March) and autumn (March to June) generations, each takes only about 6 weeks to go through development under the high temperatures prevalent during this part of the year. Emergence in summer and autumn occurs within 2 weeks of the completion of pupation (or adult eclosion), while emergence in spring is much longer and is governed by the ambient temperatures (Bungey, 1966). Those emerging early in the flight season (early November), would be the

adults of the over-wintered generation that has reached maturity together with their parent adults that were in hibernation. These parent adults may retain spermatozoa from previous autumn matings, while of the progeny that overwintered as adults some may have mated prior to their first emergence. Hence the spring flight that continues from late October to early December may contain a high proportion of these mated individuals, i.e. parent adults as well as progeny adults. As the breeding season advances from spring to autumn generations develop at a much faster rate. Because these progeny adults emerge soon after reaching maturity, they have a relatively shorter period for pre-emergence feeding and association between the young adults. Also the evidence collected so far supports the hypothesis that the period spent under bark by individuals following their maturity is critical to the likelihood of adult mating.

Hence the decrease in the proportion of females mated in the flying population from January to March as recorded in this study (Fig 4.2) could be attributed to this factor, specially because the number of parent adults dispersing in search of new breeding sites would be expected to remain fairly constant throughout the flight season. A similar trend has been observed as shown in Table 4.1 for those emerging from logs collected in the field at different times of the emergence period. However, the findings of this study demonstrate that only a small percentage of progeny matings occur prior to emergence, in temperatures resembling summer at Mt Crawford (Table 4.2). This result further suggests that a major proportion of the mated females in the flying population, as well as those emerging from logs, as recorded for the summer period, would be

Is this true?
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in Fla.

parent adults. However, when mature progeny adults are forced to remain for longer periods under bark due to unsuitable temperatures prevalent during late autumn, winter and early spring, a greater proportion of progeny adults would be expected to mate. This again is consistent with the results of this study as shown in Fig 4.2.

The very high incidence of mating observed among progeny adults reared in bark sandwiches over unusually longer periods of time (which is unlikely to occur naturally), may be an enhanced effect of what happens in natural situations. Nevertheless, it clearly demonstrates the effect of an increased period of close proximity between mature progeny adults on the incidence of mating. One of the key factors governing emergence of bark beetles are : the availability of food and population density, but in the absence of food shortages and population pressures, emergence is governed by ambient temperature, the threshold being about 22°C. Satisfactory host conditions with high attack density may result in a high natural progeny-population density of 9 adults/sq cm of bark (Bungey, 1966). Such a close proximity may provide greater opportunities for mating to occur. Observations on mating behaviour within bark sandwiches have shown that, the close association between the sexes within bark sandwiches result in consistent courting behaviour, not seen in emergents held at high density in petri dishes.

The possibility of natural mating among siblings has been indicated by the high incidence of sibling-mating in populations reared in bark sandwiches. The possible significance of such matings is not clear; perhaps even a small proportion of sibling mating could help

maintain a useful gene pool by allowing preservation of the genotype of a population that for some reason was prevented from outbreeding. At the same time, as adult females mate more than once, it is possible that a sibling-mated female could nevertheless subsequently lay eggs fertilised by spermatozoa from non-sibling matings.

Since no difference was observed in the male acceptance behaviour of mated and virgin females, both mated and unmated females could be expected to respond to the male produced aggregation pheromone in much the same way. This effect was evident during the course of this study where both types of females were caught on ipsenol baited traps. However, so far no laboratory study had been carried out to test the response of mated and unmated female I. grandicollis to male frass or to ipsenol.

The results of this study and the conclusions drawn from it pose certain questions that require further examination. The possibility that mature progeny may mate prior to emergence has a great bearing on studies concerned with the time of pheromone production as well as on the response of individuals to it. The initiation of separate attacks upon hosts by these mated females followed by development of breeding sites in the absence of males is another factor of interest. This possibility has been confirmed by the studies of All and Anderson (1972).

These possibilities and propositions may have to be incorporated into any future Ips population models and hence need due consideration. Based on the data presented herein a revised sequence of behaviour pattern has been suggested for I. grandicollis (Fig 4.3). Whether such a sequence of behaviour holds true for other species in the genus Ips needs to be investigated.

Fig 4.3 Generalised sequence of behaviour patterns of bark beetles by Cobb et al (1968) with proposed adaptations to Ips grandicollis.

General Adults

[Undergo a series of colour changes and feeding during which sexual maturity is attained.]

Mature Adults

[Mating either between siblings or non-siblings from an adjacent gallery.]

Emergence and Dispersal

[Under suitable environmental conditions.]

Host Selection

[Generally by males, but mated as well as virgin females may initiate galleries.]

Aggregation

[In response to host and male-produced attractants]

Establishment

[Mating followed by oviposition of virgin females
oviposition of already-mated females]

✓
✓

SECTION 5

RESPONSE OF I. GRANDICOLLIS MALES TO GALLERIES INITIATED
BY FEMALES

5. RESPONSE OF I. GRANDICOLLIS MALES TO GALLERIES INITIATED BY FEMALES .

5.1 Introduction

Ips grandicollis is a typical polygamous bark beetle due to the behaviour pattern followed during host colonisation.

In Ips species, males usually initiate galleries on host trees in which they construct typical male galleries called nuptial chambers (Fig. 5.1.C). During the construction of these initial galleries, males are known to produce the aggregation pheromone - ipsenol (Vité and Renwick, 1971), which together with volatiles produced by host trees, attract both sexes to selected trees. The males are subsequently joined by responding females that enter galleries initiated by males. Each male has been observed to accept up to 5 females into its nuptial chamber, wherein mating takes place. Following mating, females construct galleries that radiate from the nuptial chamber. Deposition of eggs along these galleries is an indication of successful establishment, following initial boring. The type of gallery produced are somewhat typical of the species (Chamberlin, 1939); that for I. grandicollis is shown in Fig. 5.1.A.

However, there have been reports that the females of Ips spp. in natural situations may also initiate and construct galleries. Anderson (1948) reported that females of Ips pini kept in cages without males, initiated galleries in logs.

Wilkinson (1964) found that female I. grandicollis attracted to pine logs artificially infested with males, did sometimes construct ^{solitary} galleries and produce offspring. Morgan (1967) noted that during dispersal, I. grandicollis females occasionally made short irregular galleries from which they emerged shortly after attack. Field observations of All and Anderson (1972) revealed that females of I. grandicollis frequently initiate attack and that the percentage of attacks initiated, increased progressively throughout the summer, reaching a peak in autumn. Moreover, the galleries constructed by these females were found to be of two types; virgin females constructing short irregular winding galleries 2.5 to 7.5 cm long, while mated ovipositing females constructed galleries that were 10 to 20 cm long, straight and packed with borings for 1/4 to 1/2 of their length. This packing of female galleries is probably due to the absence of males which maintain clear galleries in natural infestations.

Observations on the mating behaviour of I. grandicollis in S. Australia

✓ indicate that ^{some} females mate prior to their first emergence from the host in which they developed to maturity (see Section 4). ~~This finding casts doubt upon the general observation that most or all of the females are virgins when they first emerge.~~

~~that females initiate galleries upon hosts and mate before their first emergence.~~ These two records:

~~that females initiate galleries upon hosts and mate before their first emergence.~~ ^{That females sometimes} and that solitary females initiate galleries...

together means that such beetles may develop galleries and produce offspring in suitable hosts, without the prior presence of males in their galleries.

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This possibility raises the interesting questions:-

- i) whether males will enter galleries initiated by either virgin or mated females; and
- ii) whether mated dispersing females attracted to hosts selected by males, would still initiate galleries or would enter those of an established male.

Consequently studies were designed:-

- a) to clarify the roles of mated and virgin females in host selection and gallery initiation, in the presence or absence of galleries initiated and occupied by male beetles; and
- b) to determine the response of males to galleries initiated by both virgin and mated females.

5.2 Methods and materials

Logs of the same length and diameter were selected for the experiment to obtain a comparatively constant surface area.

These logs were subjected to the following treatments :

- a) Untreated controls.
- b) Baited with ipsenol.
- c) Infested with 10 marked males.
- d) Infested with 10 mated marked females.
- e) Infested with 10 virgin marked females.
- f) Infested with 10 pairs of marked males and females.

All treatments were replicated 5 times.

To artificially infest logs with a known number and combination of beetles I used a modification of the method of Wood et al, (1966). Entrance holes were made in the bark with a hand drill

and beetles used in each treatment were marked with a different colour of nail-varnish and released on to the appropriate logs. All logs were caged separately. Virgin females were obtained by segregating the progeny produced in bark sandwiches or logs, and rearing the females on chopped-up bark until maturity. Mated, ovipositing females were selected from mature females that emerged in the laboratory from field-infested logs. They were provided with fresh logs or put into bark sandwiches and ovipositing individuals were removed, marked and used directly in the treatments.

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One set of treated logs was exposed to a natural population of I. grandicollis in a P. radiata plantation at Mt Crawford, 60 km N.E. of Adelaide, during mid-summer of 1979. Both controls and treatments were tied on to the boles of trees about 50 m apart. All treatments and controls were distributed randomly among trees selected for their uniformity. Another set of logs that were treated in the same manner was exposed to a population of beetles emerging in the laboratory from field-infested logs. Each log was caged with logs bearing emergent beetles. Constancy in the number and sex-ratio of the emergent beetles was attempted by selecting field logs according to the density and stage of development of the beetles under bark and similarity in the surface areas of logs.

Loc. of trees in stand?

Both sets of logs were examined after one week of exposure, to determine the numbers and sex of beetles that had :

- a) Responded to treated logs .
- b) Initiated new galleries.
- c) Entered galleries already initiated by the resident beetles.

As the beetles already present in the logs bore recognisable markings, the responding population was easily identified from the resident population. Therefore only the responding unmarked beetles were included in the counts. Also the galleries initiated by the marked beetles were circled at the entry points, to distinguish such galleries from those initiated by the responding beetles.

Assessment : Galleries occupied by a single male or a male in association with females were considered as initiated by males. However, the possibility that a gallery occupied by a responding male and a female may have been initiated by a female, was not ruled out. A gallery occupied by a female only was scored as a female-initiated gallery. Short winding galleries devoid of any eggs were counted as galleries initiated by virgin females, while straight, long galleries bearing eggs were counted as those initiated by mated females (All and Anderson, 1972).

5.3 Results

In the logs subjected to various treatments and exposed to the laboratory and field populations of I. grandicollis, gallery initiation by both sexes was observed. However, in all treatments, the proportion of galleries initiated by males far exceeded those initiated by females (Table 5.1). Also no significant difference was observed between the proportion of galleries initiated by mated females and by virgin females.

The number of males and females that were attracted to the logs, and the numbers that subsequently initiated separate

Table 5.1

Gallery initiation by field and laboratory populations of I. grandicollis males and females in P. radiata logs (Results from 6 treatments with 5 replicates/treatment)

Population	% male initiated galleries $\bar{x} \pm \text{S.E.}$	% virgin female initiated galleries $\bar{x} \pm \text{S.E.}$	% mated female initiated galleries $\bar{x} \pm \text{S.E.}$
Field	93.4 \pm 1.8 (a)	2.6 \pm 0.62 (b)	4.0 \pm 1.2 (c)
Lab	93.9 \pm 0.9 (a)	2.2 \pm 0.5 (b)	3.9 \pm 0.6 (c)

Mean values followed by different letters significant at P = 0.05

galleries differed with the treatments. These responses of the field and caged populations to the experimental treatments, were analysed separately and are given in Appendix 5.1. In each experiment the 6 treatments were compared in a separate analysis of variance for each of the 3 responses that were tested, i.e. galleries initiated by males, galleries initiated by females and galleries in which females had joined males. In the field experiments, treatments were significant at $P = 0.01$ for all the 3 responses tested. Similarly, the responses of the caged beetles were significant at $P = 0.01$ for the different treatments compared, except for galleries initiated by females where the treatments differed significantly at $P = 0.05$.

The means of these 3 responses for each of the 6 treatments using the L.S.D. method, gave consistent results for both field and laboratory populations (Tables 5.2 and 5.3). In logs baited with ipsenol, a two-fold increase in the number of galleries initiated by females occurred as against logs bearing males. Nevertheless, this treatment had no effect upon the number of females entering the nuptial chambers initiated by males, since the usual polygamic ratio was maintained in all the galleries examined.

Females that entered male-initiated galleries constructed normal egg galleries that radiated from nuptial chambers. The few females that initiated their own galleries in all the treatments, were found to construct galleries similar to that described by All and Anderson (1972) for mated ovipositing individuals and for virgin females shown in Figs. 5.1.A and 5.1.B

Table 5.2

Response of field populations of I. grandicollis to P. radiata logs subjected to various treatments.

Treatment	Male initiated galleries/ replicate $\bar{x} \pm \text{S.E.}$	Females that joined males in nuptial chambers/replicate $\bar{x} \pm \text{S.E.}$	Female initiated galleries/ replicate $\bar{x} \pm \text{S.E.}$
Control	2.4 \pm 0.51	5.6 \pm 0.51	0.4 \pm 0.24
Ipsenol	30.6 \pm 2.4	67.8 \pm 6.87	2.8 \pm 0.37
Male infested	40.4 \pm 3.83	61.2 \pm 4.77	1.2 \pm 0.37
Mated females	7.0 \pm 0.71	5.6 \pm 1.67	0.4 \pm 0.24
Virgin females	15.8 \pm 1.16	6.6 \pm 1.47	1.0 \pm 0.45
($\sigma^7 + \rho$) pair	22.4 \pm 2.21	47.6 \pm 4.03	0.8 \pm 0.37
LSD	9.30	28.22	1.16

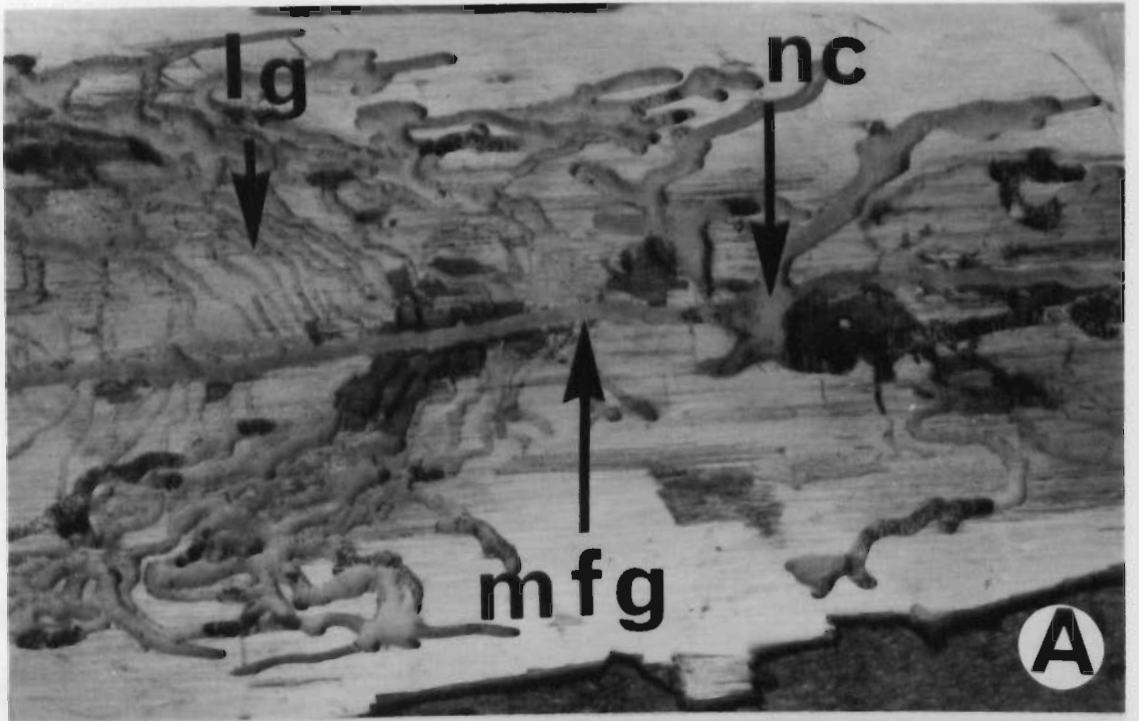
Table 5.3

Response of laboratory population of I. grandicollis to
P. radiata logs subjected to various treatments.

Treatment	Male initiated galleries/ replicate $\bar{x} \pm \text{S.E.}$	Females that joined males in nuptial chamber/ replicate $\bar{x} \pm \text{S.E.}$	Female initiated galleries/ replicate $\bar{x} \pm \text{S.E.}$
Control	4.6 ± 0.7	8.0 ± 2.2	0.4 ± 0.24
Ipsenol	28.6 ± 1.9	20.6 ± 1.96	2.0 ± 0.31
Female infested	26.2 ± 1.11	33.2 ± 5.7	0.8 ± 0.5
Mated females	10.2 ± 0.97	5.2 ± 0.9	0.4 ± 0.30
Virgin females	8.2 ± 2.1	5.6 ± 1.2	0.8 ± 0.4
(♂ + ♀) pair	12.6 ± 1.1	19.2 ± 0.9	0.6 ± 0.40
LSD	3.17	8.45	1.10

Fig 5.1.

- A. A typical gallery constructed in P. radiata slash by I. grandicollis male and females. The gallery is initiated by the male that constructs the nuptial chamber (nc) from which radiate the straight gallery (mfg) made by the ovipositing females. Also, note the larval galleries (lg) that run approximately perpendicular to the female gallery.
- B. A gallery initiated by a virgin female of I. grandicollis in a P. radiata log, exposed to a dispersing field population. Note the short, winding gallery that is devoid of any egg niches.
- C. Typical galleries initiated and constructed by I. grandicollis males in a P. radiata log. Note the nature of the nuptial chamber; short and broad.



respectively. This behaviour pattern occurred despite the presence of adjacent male-initiated galleries.

Both males as well as females from the field and laboratory populations responded to the presence of females in the test logs, although the numbers attracted were relatively small (Table 5.4). Also some of the males thus attracted had entered galleries initiated by females. Thirty percent of the virgin females that initiated galleries were joined by the responding males, while only 11 per cent of the mated ovipositing females were found in association with a male.

On close examination, the short winding galleries initiated by virgin females were found to be clear of any boring dust that is usually present in egg galleries. However, a few of the galleries made by ovipositing females were also of the same type. Depending on whether the entrance to the gallery was blocked by frass or not, a difference in the male entry behaviour pattern was observed. Males after encountering galleries with unblocked entrances, had constructed a nuptial chamber at the very base of the female gallery. This was the case with the galleries constructed by virgin females (Fig. 5.2.B) and some of the galleries constructed by mated ovipositing females (Fig. 5.2.C) and subsequently joined by males.

Most of the galleries constructed by mated ovipositing females had blocked entrances and no males had entered them. Instead the males had constructed a separate nuptial chamber adjacent to the base of the female gallery (Fig. 5.2.A).

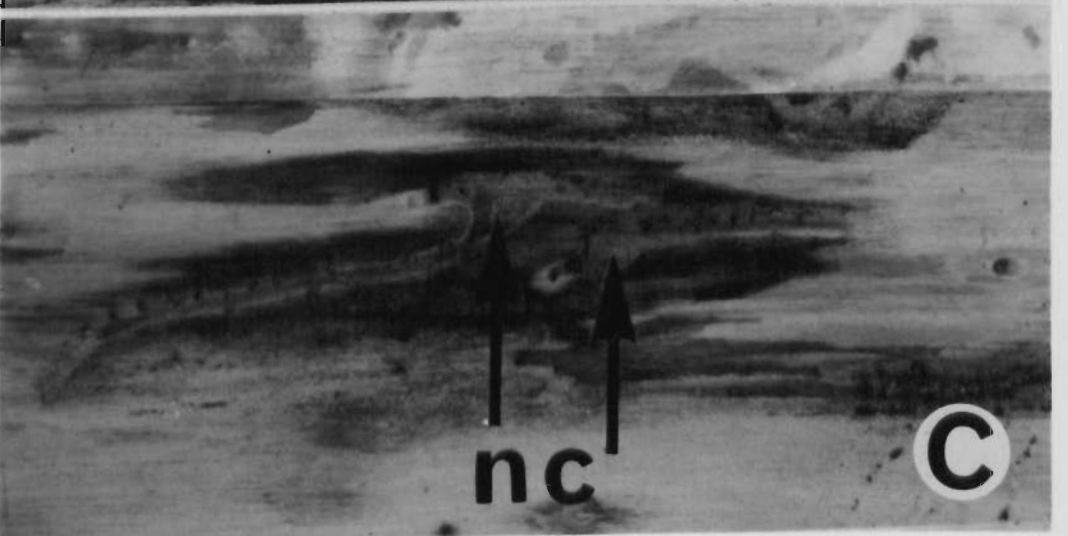
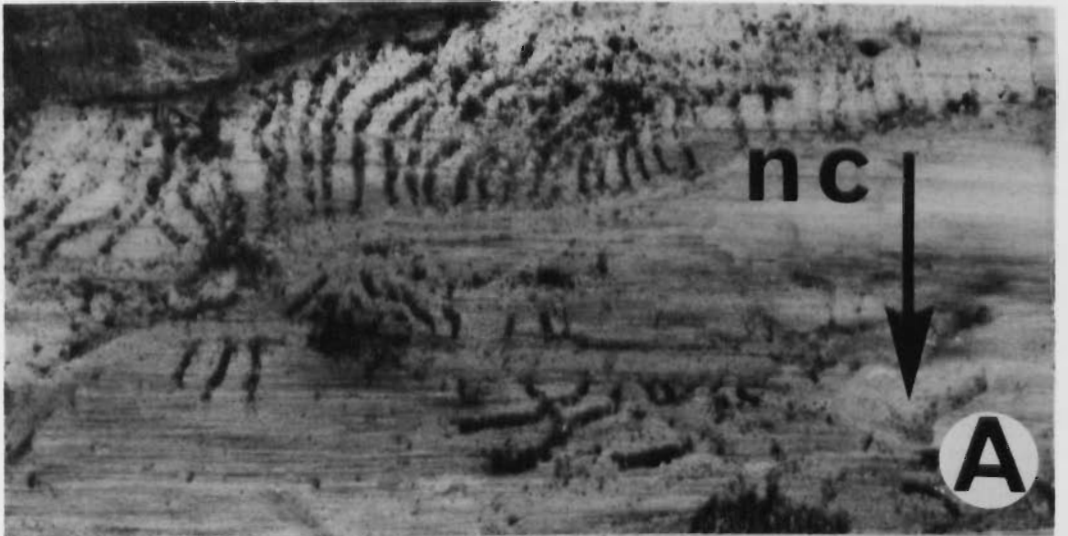
Table 5.4

Response of male I. grandicollis to galleries initiated by virgin and mated females. (No of replicates/treatment = 5; No. of galleries examined/replicate = 10.)

Initiator of gallery	Responding population	Male initiated galleries/ replicate $\bar{x} \pm \text{S.E.}$	% of female galleries entered by males $\bar{x} \pm \text{S.E.}$
Mated females	Field	7.0 \pm 0.7	12.0 \pm 0.9
	Lab	10.2 \pm 0.9	10.0 \pm 0.8
Virgin females	Field	11.8 \pm 1.1	34.0 \pm 3.1
	Lab	8.2 \pm 0.7	24.7 \pm 2.0

Fig 5.2.

- A. A gallery constructed in a P. radiata log artificially infested with ovipositing I. grandicollis females and exposed to a dispersing field population. Note the nuptial chamber (nc) constructed alongside the female initiated egg gallery. Also note the two galleries have separate entrance holes.
- B. A gallery in a P. radiata log, artificially infested with virgin I. grandicollis females and exposed to a dispersing natural population. Note the nuptial chamber (nc) at the base of the virgin female initiated gallery similar to that seen in Fig. 5.1.B.
- C. A gallery in a P. radiata log, artificially infested with ovipositing I. grandicollis females and exposed to a dispersing natural population. Note the nuptial chamber (nc) at the base of the female gallery, also the egg niches along the gallery.



5.4 Conclusions and discussion

The findings of All and Anderson (1972) for the existence and types of both virgin and mated female initiated galleries were supported. The fact that this behaviour pattern was observed even in the presence of male-initiated galleries, contradicts to some extent, earlier observations on the behaviour of Ips species. Females of this species are known to be attracted to male-initiated nuptial chambers due to the production and release of the aggregation pheromone. There can be little doubt that most females in the dispersing population actually join males occupying nuptial chambers.

Nevertheless, why even a small percentage of the population behave otherwise, raises two interesting factors

- i) that the initiation of galleries on hosts by females is not dependent upon the presence of males or the aggregating pheromone, and
- ii) that even in the presence of males and ipsenol, females may initiate galleries.

The fact that males respond to and enter some galleries initiated by females, raises another question : whether males respond to virgin females or to mated females or to both. The response of males to virgin females that initiate galleries could be of biological significance because such females are considered lost to the population, unless they are subsequently either joined by males or enter male occupied galleries.

Males that respond to mated females in their galleries are initially, at least, superfluous. A previous experiment (see Section 4.2.8) resulted in both mated and virgin females being accepted into nuptial chambers by resident males, and moreover by the same male. Thus within the field situation, opportunity for all females to mate and to oviposit is dominated by the typical polygamous

relationship and the deviations discussed here are probably temporary and overcome by the acceptance behaviour of both sexes.

Females may produce more than one batch of eggs in the same or in different trees of their host species and therefore, could be expected to initiate galleries in some of these instances. In those cases where mated females are accepted into male occupied nuptial chambers, repeated mating might be a prerequisite for the production of more than one egg batch per female, whereas, where females once mated continue oviposition in the absence of males, fewer egg batches may be laid. This comparison has not been investigated for I. grandicollis; though it is known from rearings in bark sandwiches that a mated female will construct her own gallery and produce offspring (see Section 5). Deposition of viable eggs in a second gallery without re-mating has been demonstrated for Dendroctonus monticolae (Reid, 1958) and it would appear that this is also likely for I. grandicollis in view of the behaviour demonstrated in bark sandwiches.

SECTION 6

STUDIES ON THE AGGREGATION PHEROMONE, IPSENOL OF I. GRANDICOLLIS

6. STUDIES ON THE AGGREGATION PHEROMONE, IPSENOL, OF
I. GRANDICOLLIS

6.1 Determination of the amount of ipsenol present in
extracts of unmated and mated male beetles fed for 48 hr

6.1.1 Introduction

Aggregation pheromones of several species of Ips bark beetles have been described and ipsenol has been identified as the pheromone responsible for the aggregation of Ips grandicollis (Vité & Renwick, 1971). This compound produced in the hindgut of the male beetle following feeding in the phloem tissues of the host (Hughes, 1974), is released with the faecal pellets during defaecation (Vité et al, 1964; Pitman et al, 1965; Vité and Pitman 1968; Vité et al, 1972). Werner (1972 a,b,c) had determined the various environmental and physiological factors that influence both the production of the pheromone and the response of males and females to it.

His study demonstrated that virgin males produced attractive frass for up to 9 days after boring into logs, with frass of maximum attractiveness being produced on the second and third days of boring. However, the attractiveness of male frass gradually decreased for a period of 4 days after mating, with frass collected 1 day after mating being most attractive. In the present study an attempt has been made to quantify the amount of ipsenol present in extracts of both virgin and mated males that had fed for 48 hr.

In the past, concentrations of ipsenol in gut extracts of

beetles had been expressed in terms of GC peak heights (Hughes, 1974; Hughes and Renwick, 1977). Without response factors for individual studies it is not possible to measure the absolute amount of ipsenol present or to compare the amount of ipsenol in different beetles. Recently Byers et al (1979) determined the quantities of ipsenol and ipsdienol in Ips paraconfusus, by comparison of GLC peak areas with known amounts of the synthetic compounds.

In the present study an attempt has been made to develop a better method to quantify the amount of ipsenol present in extracts of whole beetles, by introducing a suitable internal standard in the solvent used for the extraction of the pheromone from the beetles. The quantity of ipsenol in extracts of beetles was then determined by comparison of the ratio of GC/MS peak areas using a standard curve obtained with the internal standard and known amounts of the synthetic compound. The authenticity of the ipsenol in the extracts of beetles was also confirmed by its GC/MS cracking pattern.

6.1.2 Materials and methods

Beetles at a fairly lightly pigmented stage were extracted from galleries in field-infested logs or from bark sandwiches. The males thus obtained were considered unmated (see Section 4) and were used directly in the experiment. Female beetles were released into fresh logs bearing males that had constructed nuptial chambers. After 24 hr, the males that were in association with females in nuptial chambers were extracted and used as a source of mated males.

Both virgin and mated males were allowed to feed separately

for 48 hr in logs of P. radiata and they were then removed and held at -4°C until GC/MS analysis.

Groups of 10 beetles were thawed, weighed and transferred to glass tubes in ice to be crushed in 0.2 ml of the solvent, methylene chloride containing $5\ \mu\text{g}\cdot\text{ml}^{-1}$ of menthol, the internal standard. These extracts were analysed on a GC/MS for the quantification of ipsenol.

6.1.3 Instrumentation

GC/MS single ion monitor analysis was carried out using a Hewlett Packard Model HP 5992 B system, with a membrane separator and an electron multiplier (EM) detector. Electron impact (70 EV) mass-spectra were obtained after calibration with perfluoro-tributylamine (PFTBA). A 74 cm x 2 mm i.d. glass column packed with a liquid phase of 2 per cent OV 101 + 0.2 per cent carbowax x 20 M on a support of 100/120 mesh chromosorb W- HP was eluted, with helium as carrier gas at a flow rate of $24\ \text{ml}\cdot\text{min}^{-1}$. The GC was operated isothermally at 64°C .

The identity of the test compound (ipsenol) in the insect extract was confirmed by comparison with a solution of the synthetic ipsenol (Borregarrd Co.), using : (a) a T.L.C. column, (b) GC retention times, (c) the 10 most significant peaks in the spectra, and (d) the literature mass spectral data obtained from a benzene extract of frass of I. confusus which had fed on Pinus ponderosa (Silverstein and Rodin, 1966). One hundred males fed for 48 hr were crushed in 2 ml of methylene chloride. This extract was placed on a silica-gel column and eluted with 5 per cent methanol-petroleum spirit (B.P. 30° to 40°C). The eluent thus obtained was used in the above comparison.

6.1.4 Results

Comparison of the data of the three spectra obtained from (a) gut extracts of I. grandicollis (b) synthetic ipsenol and (c) data from frass extracts of I. confusus, using the ten most significant peaks (Table 6.1), gave the comparison shown in Fig 6.1.1 which is expressed in the form of a Similarity Index (S.I.).

Results of the quantification of the pheromone from different extracts of mated and virgin beetles fed for 48 hr, using the standard curve (Appendix 6.1) is shown in Table 6.1.1. The mated males contained a mean ipsenol concentration of $13.7 \pm 1.1 \times 10^{-9}$ g (range 5.0×10^{-9} g to 17.5×10^{-9} g) per beetle while the unmated males contained a mean concentration of $17.1 \pm 6.9 \times 10^{-9}$ g (range 7.4×10^{-9} g to 51.3×10^{-9} g). Comparison of the means using the t-test indicated that the two treatments were not significantly different at $P = 0.05$ (Table 6.1.2).

6.1.5 Discussion and conclusion

✓ Ips ^aparconfusus _^ males exposed to various concentrations of myrcene vapour for 18 ± 1 hr produced a maximum concentration of 37.3×10^{-8} g of ipsenol per beetle (Byers et al, 1979). However, I. grandicollis males feeding for 48 hr produced a maximum concentration of 51.3×10^{-9} g of ipsenol per beetle. Because Byers et al (1979) studied ipsenol production under a rather artificial situation of exposure of beetles to the precursor compound in the vapour phase, their results cannot be compared

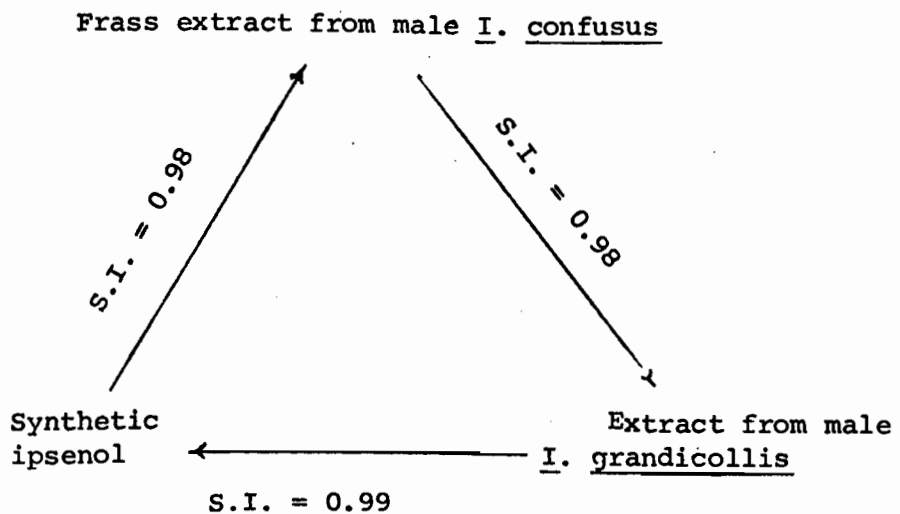
Table 6.1

Mass spectral data (relative intensities) of the ten most significant* peaks for ipsenol obtained from three different sources.

m/e	Abundance (per cent of base peak)		
	Frass extract <u>I. confusus</u>	Gut extract <u>I. grandicollis</u>	Synthetic ipsenol
39	37	28	31
41	70	63	68
43	66	41	44
45	26	25	22
53	25	17	19
57	17	21	22
67	35	48	54
68	<u>100</u>	<u>100</u>	<u>100</u>
69	64	71	77
85	15	41	20

* Significance = mass x abundance

Fig 6.1.1 Comparison of the three spectra for ipsenol obtained from different sources using the Similarity Index (S.I.). A retention time of 2.4 min was recorded for the 2 samples of ipsenol (a) synthetic compound (b) extracts from male I. grandicollis.



$$S.I. = \frac{\sum_{m=1}^{1000} A_m \cdot a_m}{(\sum_{m=1}^{1000} A_m^2 \cdot \sum_{m=1}^{1000} a_m^2)^{\frac{1}{2}}}$$

where A_m = abundance of ion at mass "m" in unknown
 a_m = abundance of ion at mass "m" in library spectrum

Table 6.1.1

Amount of ipsenol present in extracts of male I. grandicollis
fed for 48 hr.

Condition of the male	Wt of 10 males (10^{-3} g)	Amount of ipsenol/ 10 males (10^{-9} g)	Amount of ipsenol/male (10^{-9} g)
Mated	35.2	119.5	11.9
	35.9	143.0	14.3
	36.0	144.7	14.5
	36.4	145.4	14.5
	37.0	175.2	17.5
	34.9	95.2	9.5
Virgin	33.0	87.4	8.7
	37.5	101.52	10.1
	36.5	96.1	9.6
	39.5	153.2	15.3
	48.5	512.5	51.3*
	33.0	74.1	7.4

* Note the exceptionally high value for this replicate.

Table 6.1.2

Relationship between the weight of I. grandicollis males fed for 48 hr and the concentration of ipsenol in their extracts

Condition of the male	No. of males	Weight/male (10 ⁻³ g) $\bar{x} \pm \text{S.E.}$	Ipsenol/male (10 ⁻⁹ g) $\bar{x} \pm \text{S.E.}$	Ipsenol/g of male (10 ⁻⁶ g) $\bar{x} \pm \text{S.E.}$
Mated	60	3.6 ± 0.03	13.7 ± 1.1	3.8 ± 0.29
Virgin	60	3.8 ± 0.22	17.1 ± 6.9	4.1 ± 1.31

Mean values for mated and virgin males are not significantly different at $p = 0.05$.

with those of the present study. Also, their study may have been carried out under optimum conditions for pheromone production (hence the disparity in the magnitudes) which would not be encountered by beetles under natural situations.

For comparison of amounts of pheromone present in test beetles subjected to various treatments, the expression of the pheromone concentration per weight of beetle was found to be a better criterion (Table 6.1.2). Such a measure would remove the effect of beetle size on the amount of pheromone produced and hence permit comparison between other bark beetles.

The presence of similar amounts of pheromone in extracts of mated and unmated I. grandicollis males that fed for 48 hr, indicate that mating apparently has no effect on the production of ipsenol. Vité and Gara (1962); Gara (1963); and Vité and Pitman (1969) reported that polygamous I. paraconfusus males continued to produce the attractant regardless of mating. In contrast, Borden (1967) found that mating with at least three females suppressed the production of attractive frass by I. paraconfusus.

However, findings of Wilkinson (1964); Ward (1967) and Werner (1972b) indicate a decrease in the attractiveness of frass produced by male I. grandicollis with mating. Nevertheless, the detection of an exceptionally large amount of ipsenol in one of the extracts of fed, virgin male I. grandicollis (Table 6.1.1) demonstrates the variability in the production of ipsenol in beetles, even when subjected to the same treatment. The technique described above was used to examine the time when ipsenol is first produced by boring male beetles (see Section 6.2).

6.2 Determination of the minimum period required by boring adult *I. grandicollis* males to produce ipsenol

6.2.1 Introduction

Frass of adult bark beetles includes both faecal pellets or excrement that represent the undigested food and boring dust (chewed up pieces of bark) that does not pass through the gut (Hopf, 1937).

Initial studies on the attractancy of frass produced by bark beetles have indicated the source of attraction to be localized in the excrement (Wood and Bushing, 1963; Wood et al, 1966).

As the bulk of the frass consists of chewed up pieces of bark, only the frass containing faecal pellets will contain any ipsenol. Thus the initial appearance of faecal pellets in the frass of a male beetle is likely to coincide with the first release of ipsenol to its outside environment. Such an inference, however, depends on the propositions:- (a) that the first faecal pellets produced by any male *I. grandicollis* will contain ipsenol, and (b) that the precursor compound is ingested during initial boring and metabolised into ipsenol. These two propositions may be tested (i) by a bioassay of the faecal pellets when they first appear in the frass and (ii) by analysis of gut extracts of males that had bored into logs for varying periods. Hence this study was designed to (1) determine the earliest time from commencement of boring into suitable host material that faecal pellets would appear in frass of male beetles and (2) determine the earliest

time that ipsenol would be present in the gut of such beetles.

6.2.2 Methods and materials

- a) Beetles were collected on emergence from logs held in cages, or were extracted directly from their galleries. Males were selected and released on to P. radiata logs with prepared entrance holes in the bark. The frass produced by each beetle was collected into a gelatin capsule placed over the entrance hole, and examined at intervals for the presence of faecal pellets.
- b) Males, obtained as in (a) were released into logs. After they had bored for varying periods from 3 to 48 hr, batches of 40 beetles were removed from their galleries. One half of each batch was frozen immediately, while the other was left for a period of 12 hr before being frozen. Each batch of 20 beetles was divided into groups of 5 and tested for the presence of ipsenol using the method described in Sections 6.1.2 and 6.1.3.

6.2.3 Results

- a) Examination of the frass showed that faecal pellets were present from 6 to 9 hr from entry into prepared entrance holes (Fig 6.2.1). Thereafter the number of faecal pellets in frass increased markedly reaching a cumulative total of 95 pellets per beetle after 48 hr of boring.
- b) On analysis of beetle extracts, ipsenol was first detected in beetles that had bored for 9 to 12 hr (Fig 6.2.2). Extracts

Fig 6.2.1 Relationship between duration of boring and cumulative number of faecal pellets in frass of male I. grandicollis.

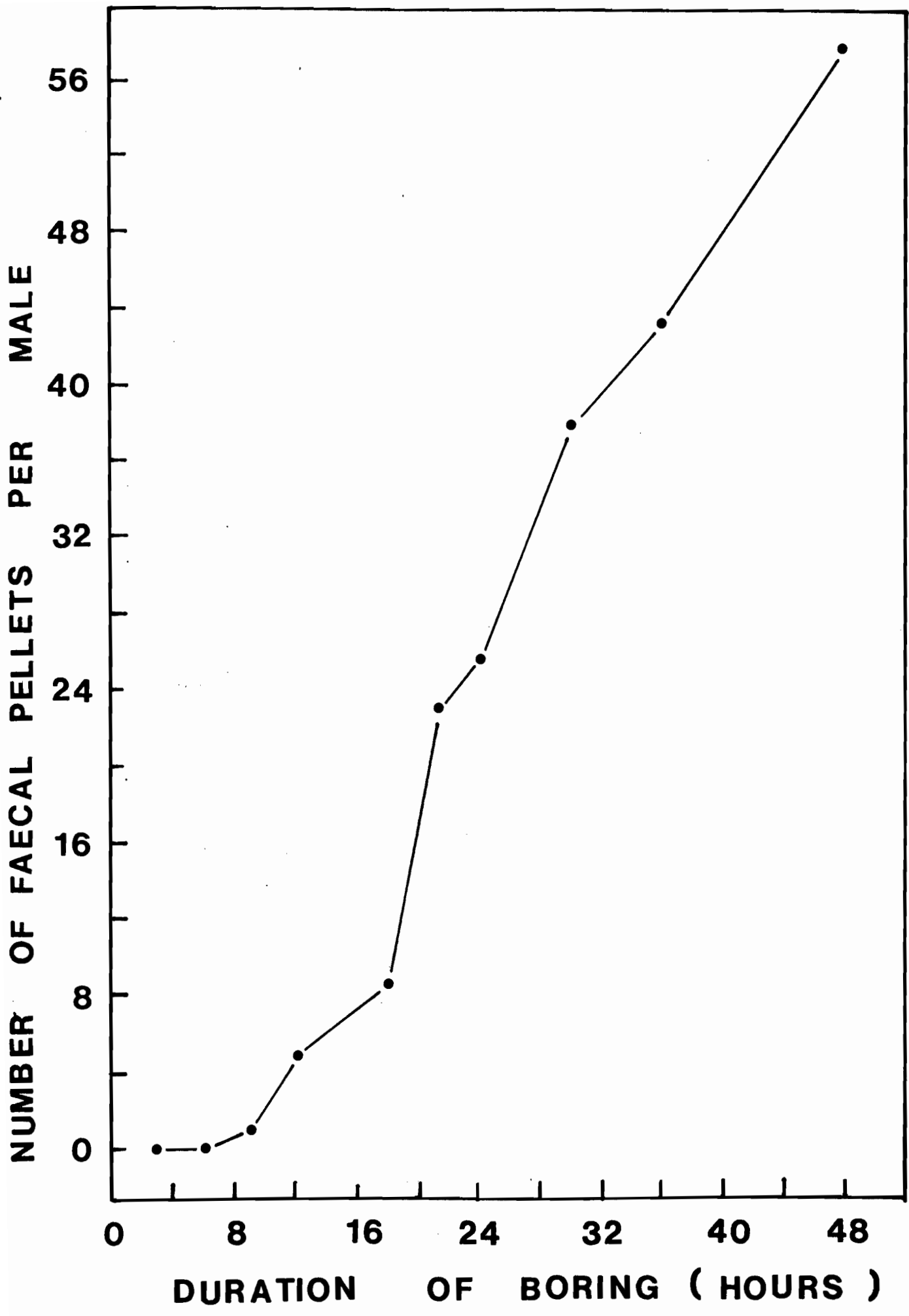
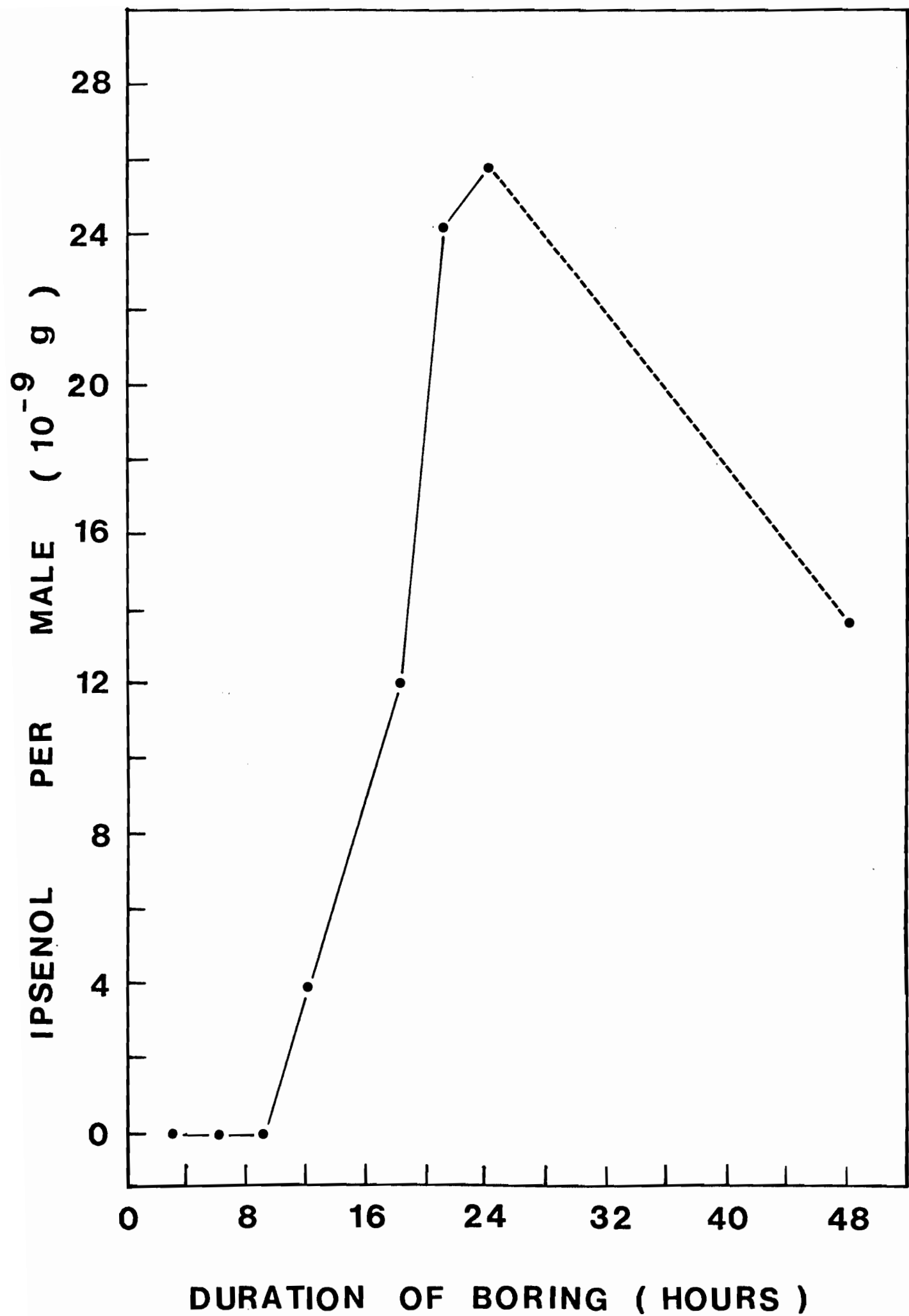


Fig 6.2.2 Relationship between duration of boring and concentration of ipsenol in extracts of male I. grandicollis.

●-----● = No observations made between 24 and 48 hr.



of beetles that had bored for shorter durations, gave a strong peak at m/e 136 with a retention time of 1.4 min. The concentration of this compound was observed to decrease as ipsenol increased in the extracts. Examples of raw data on GC/MS analysis of extracts of males that had bored for 8 hr and 18 hr are shown in Figs 6.2.3 and 6.2.4 respectively. The possibility that this compound of R.T. 1.4 min, is a precursor for ipsenol has not yet been investigated in detail, but myrcene, myrcenol and ipsdienol were excluded on the basis of their mass spectra and retention times.

A maximum ipsenol concentration of 25.75×10^{-9} g/male was recorded for males that had bored for 24 hr. However, those that had bored for 48 hr contained ca. 14×10^{-9} g of ipsenol/male.

Ipsenol was not detected in males that had bored for varying periods and thereafter left for 12 hr.

6.2.4 Discussion and conclusion

The appearance of faecal pellets in frass after 6 to 9 hr of boring indicates that food takes a minimum of 6 to 9 hr to be digested, assuming ingestion began as the males began to bore into the bark. Moreover, the appearance of ipsenol in extracts of beetles that bored for 9 to 12 hr indicates that the production of detectable amounts of ipsenol does not occur until about 3 hr after the digestion of ingested food had begun. The data supports the evidence of Hughes (1974) and Hughes and Renwick (1977) that feeding together with the distention of the crop acts as a stimulus for the production of ipsenol. Their study has proposed a generalised scheme for the neural and hormonal control of ipsenol and ipsdienol production in I. paraconfusus. From the small amounts of ipsenol produced by newly-emerged beetles, on topical treatment with JH,

Fig 6.2.3 GC/MS tracing of compounds extracted from male

I. grandicollis that had bored for 8 hr in

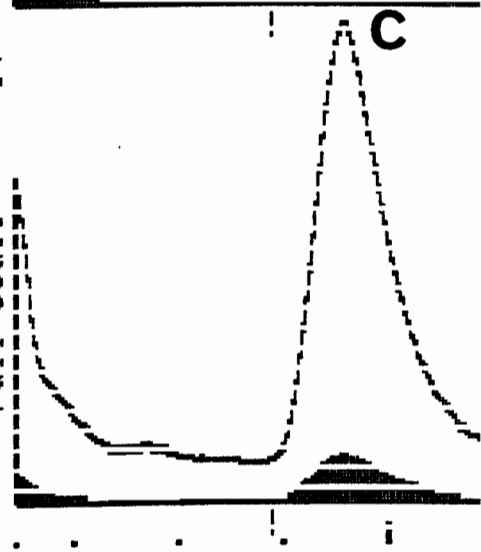
P. radiata logs.

A - unknown compound (R.T. 1.4 min)

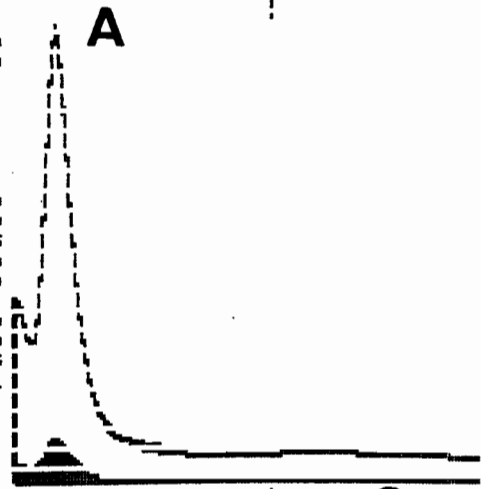
C - menthol (internal standard) (R.T. 4.2 min)

Note that ipsenol (R.T. 2.4 min) is below the level of detection in males that had bored for 8 hr, but clearly detectable after 18 hr of boring (Fig 6.2.4).

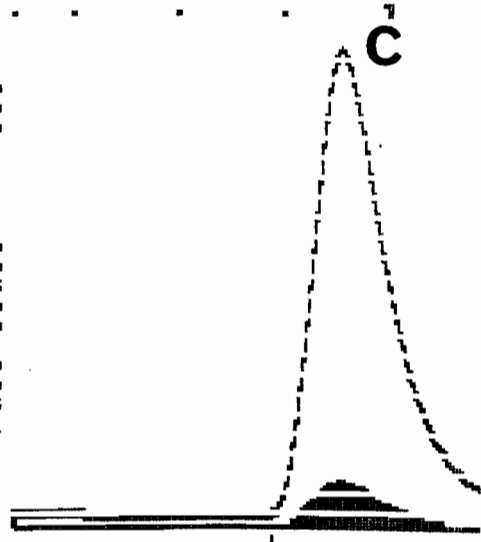
ION = 68.10
Full Scale = 64



ION = 136.10
Full Scale = 88



ION = 138.20
Full Scale = 115



INTEGRATION SENSITIVITY = 0.010 AREA THRESHOLD = 100 SMOOTHING FACTOR = 1.00

1 ION	68.10	PEAK	RET. TIME	AREA	AREA/(SUM OF AREAS)	AREA/(LARGEST PEAK)
		1	4.6	45309	100.00000	100.00000
SUM OF AREAS = 45309						
2 ION	136.10	PEAK	RET. TIME	AREA	AREA/(SUM OF AREAS)	AREA/(LARGEST PEAK)
		1	4.4	541	100.00000	100.00000
SUM OF AREAS = 541						
3 ION	138.20	PEAK	RET. TIME	AREA	AREA/(SUM OF AREAS)	AREA/(LARGEST PEAK)
		1	4.5	87689	100.00000	100.00000
SUM OF AREAS = 87689						

Fig 6.2.4 GC/MS tracing of compounds extracted from male

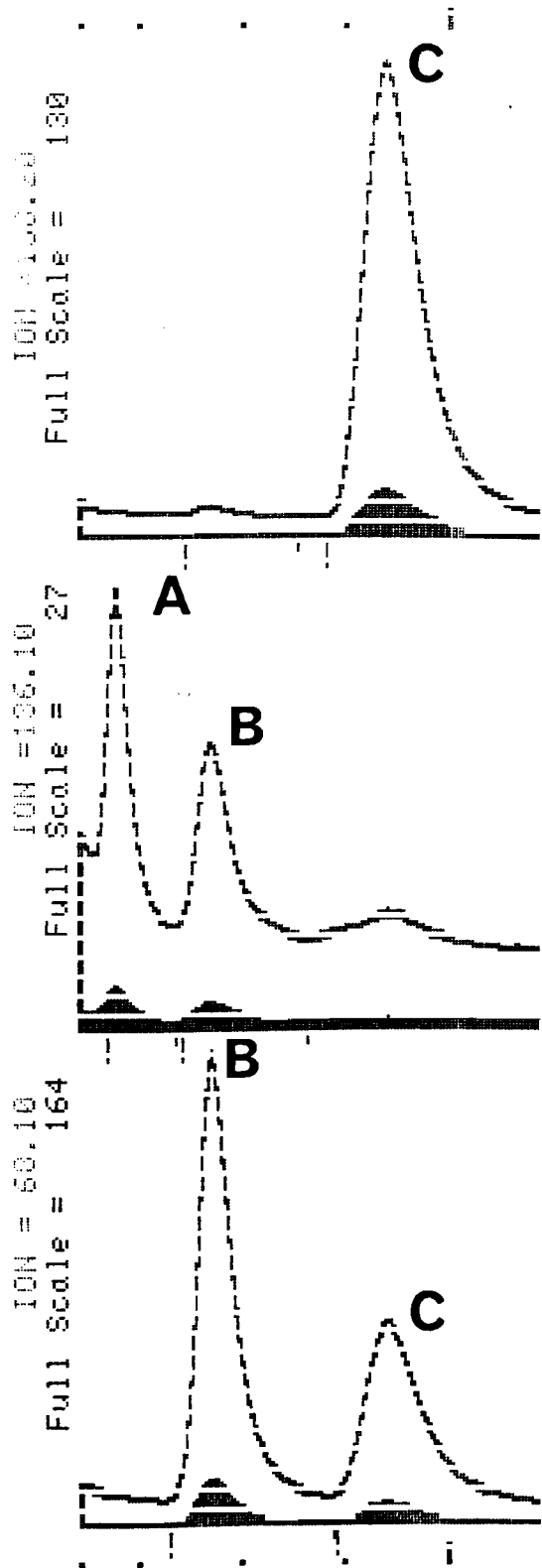
I. grandicollis that had bored for 18 hr in

P. radiata logs.

A - unknown compound (R.T. 1.4 min)

B - ipsenol ($M^+ - H_2O = 136.1$) (R.T. 2.4 min)

C - menthol ($M^+ - H_2O = 138.2$) (R.T. 4.2 min)



INTEGRATION SENSITIVITY = 0.010 AREA THRESHOLD = 20 SMOOTHING FACTOR = 1.00

ION	PEAK	RET. TIME	AREA	AREA/(SUM OF AREAS)	AREA/(LARGEST PEAK)
1 ION 60.10	1	2.7	66997	61.39137	100.00000
	2	4.4	42134	38.60863	62.88934
	SUM OF AREAS =			109131	
2 ION 136.10	1	1.8	948	18.54921	22.77352
	2	2.7	4164	81.45079	100.00000
	SUM OF AREAS =			5112	
3 ION 138.20	1	2.6	1143	1.34298	1.36126
	2	4.4	93940	98.65702	100.00000
	SUM OF AREAS =			95083	

Hughes and Renwick (1977) inferred that some pheromone precursor was present in such beetles, with additional precursor being acquired when these beetles invade new hosts.

However, the present study clearly demonstrates the presence of ipsenol in the gut only after the digestion of the food has occurred, providing conclusive evidence for the production of ipsenol on metabolism of the ingested precursor.

The decrease in the amount of ipsenol in beetle extracts after 24 hr of boring, with an increase in the number of faecal pellets after the same period, is contradictory. Such a relationship also contradicts the data (Werner, 1972c) on the maximum attractiveness of frass of I. grandicollis at 2 to 3 days after first production. However, with defaecation, beetles may have lost some of the attractive faecal pellets to the outside. This may be a factor contributing to the decrease in the amount of ipsenol in beetles after 24 hr of feeding. Such a proposition can be determined only by testing the attractiveness of the faecal pellets voided during this period.

However, Werner (1972b) has shown that in I. grandicollis, maximum attractiveness of frass (after 2 to 3 days of boring), does not coincide with maximum frass production (after 5 days of boring) although the attractiveness of the frass had not been related to the number of faecal pellets contained in the frass. On the other hand in I. paraconfusus males produced frass of maximum attractiveness 12 to 15 hr after boring (Wood and Bushing, 1963), and the attractiveness remained constant, even with a marked increase (59 to 520) in the production of faecal pellets, with 16 to 40 hr of feeding

(Wood et al, 1966).

In the light of the above observations, findings of this study provide further evidence that only those faecal pellets produced during initial feeding are attractive, thereby demonstrating that ipsenol production decreases, or may even cease, when digestion continues. Such an inference suggests inhibition of pheromone production by mechanisms other than those responsible for the stimulation of its production (Hughes and Renwick, 1977).

As none of the beetles that were starved for 12 hr after varying periods of boring contained ipsenol, it could be concluded that (a) those beetles that had produced ipsenol had lost it probably by defaecation during the non-feeding period; or (b) that continuous feeding is necessary for the production of ipsenol.

The appearance of an unknown compound with a distinctively different retention time and mass spectrum to ipsenol or to its known precursors; myrcene, myrcenol and ipsdienol, is of interest and points to the possible influence of individual host terpenes on the production of volatile materials in the gut (Hughes, 1973a,b) Renwick et al, (1976) have detected a number of alcohols, previously not found in nature, in the hindguts of Dendroctonus spp. and Ips spp. It is therefore not surprising that another compound has been detected in the extracts of I. grandicollis males. The decrease of the concentration of the unknown compound with increasing ipsenol concentration does suggest a role in the chain of events leading to the production of ipsenol.

6.3 Analysis of extracts of male *I. grandicollis*

- a) that rejected 'resistant' trees, and
- b) obtained from the dispersing natural population

6.3.1 Introduction

Studies carried out in Section 2 to test the theories of Person (1931) and Callaham (1952 in Wood 1972) on the process of host selection indicated that, in *I. grandicollis*, host acceptance occurs sometime during the initial boring period that follows random landing on trees.

This finding and the data on catches of beetles on 'resistant' and 'susceptible' trees (Table 2.1) resulted in the speculation that beetles would not produce any pheromone during test boring. Hughes (1974) reported the absence of ipsenol in gut extracts of newly-emerged males of *I. grandicollis*. Based on his findings, this study was carried out to investigate :

- (a) whether males in the dispersing population contained any ipsenol; and
- (b) whether beetles boring into 'resistant' trees contained ipsenol on retreating from such trees.

6.3.2 Materials and methods

- (a) The experiment was carried out in an infested *P. radiata* plantation in South Australia. Dispersing *I. grandicollis* were caught on sticky traps baited with ipsenol and the males were frozen and their extracts were assessed for the presence of ipsenol (See Section 6.1).
- (b) Two 'resistant' *P. radiata* trees were selected according to the risk-rating system of Mason (1966). Dispersing *I. grandicollis*

beetles were attracted to these two trees by tying wicks baited with ipsenol to their boles. Beetles attracted to the pheromone landed nearby on the bole of the tree and began to bore into it. The points of entry made into the bark by the boring beetles were covered with gelatin capsules pinned to the bark. The beetles that retreated from the entrance galleries, after a period of boring, were caught in the gelatin capsules. They were frozen and later tested for the presence of ipsenol as in Section 6.1.

6.3.3 Results

- (a) GC/MS analysis did not detect any ipsenol in extracts of beetles that had rejected 'resistant' trees.
- (b) Extracts of batches of dispersing males contained very small quantities of ipsenol, a mean concentration of $0.99 \pm 1.98 \times 10^{-9}$ g (range 0 to 3×10^{-9} g) per beetle.

6.3.4 Discussion and conclusion

The results demonstrate that ipsenol is not produced by males if they reject sites of attack, after initial boring. It has been previously demonstrated (Section 6.2) that in 'susceptible' host material, ipsenol is produced by males, 9 to 12 hr after entering the bark, and moreover, after the ingested food has been digested. The data from both experiments indicate:- (a) that beetles during

test-boring do not feed and hence cannot produce ipsenol, and (b) that the message to retreat from 'resistant' trees is perceived on or before reaching the inner bark tissue.

Inner bark tissue is known to be the source of food as well as the source of the pheromone precursor for Ips bark beetles (Pitman, 1966; Vité et al, 1972; Hughes, 1974). The second proposition appears probable from earlier results (Section 3) where beetles entering resistant trees bored upto the phloem tissue before rejecting the site of attack. The phloem tissue of 'resistant' trees produce secondary resin on injury. This perhaps is associated with, and responsible for, the beetles' rejection of 'resistant' trees and for the inability of the beetles to feed in tissues of such trees. By contrast, the absence of secondary resin production is always associated with continued boring leading to the construction of nuptial chambers and production of attractive frass.

The small amounts of ipsenol present in extracts of certain batches of dispersing males, could be due to the proportion of males that have emerged from previous sites of successful establishment. Wilkinson (1964) reported that male I. grandicollis abandoned nuptial chambers 5 days after their initiation and Werner (1972) showed that such males still produced attractive frass. In contrast, those that emerged from hosts in which they developed do not contain any ipsenol (Hughes, 1974), nor do those that retreat after boring into 'resistant' trees (Section 6.3.3.). The dispersing population is likely to contain individuals from all 3 of the above sources. Hence the presence of small amounts of ipsenol in extracts of some of the batches of dispersing males and the complete absence of it in others relate well with the known behaviour of the dispersing Ips grandicollis population.

SECTION 7

GENERAL DISCUSSION

7. GENERAL DISCUSSION

An outstanding biological characteristic of bark beetles is the comparatively long time spent within the bark of their hosts. During the short periods of activity outside the bark, dispersing beetles encounter new hosts, a factor which governs both spatial and temporal aspects of population dispersal. Yet, it is during this brief period of dispersal that the sequence of events that lead to the destruction of the host species is initiated and hence concerns the forest industry as a whole.

The present investigations into the location of suitable trees by dispersing I. grandicollis supported the theory of Callaham (1952 in Wood 1972) on host selection. The insect on landing, discriminates between individual trees within a stand of potential host trees during the initial boring activity, on the basis of the tree-vigour defined here as the ability of their phloem or inner bark tissues to produce secondary resin. Similarly Wood (1972) reported that Dendroctonus brevicomis discriminated between non-host and host as well as between 'resistant' and 'susceptible' trees within the potential host species, after landing and during test boring (i.e. the biting response). However, his study failed to indicate at what point during the initial interaction between the insect and the tree, rejection or acceptance of a tree occurred.

Oleoresin, in coniferous trees, is known to be a major factor contributing to their resistance to attack by bark beetles. Most bark beetles on encountering resin ducts in phloem and xylem tissues abandon the tree, while some succumb to the physical

effects of resin (Smith, 1961 a,b, 1963). Observations during this study indicate that failure of beetles to encounter resin ducts, or to stimulate production of secondary resin from damaged phloem tissue, as the main factors that determine the suitability of a tree for establishment. The data presented herein demonstrate that rejection or acceptance of a potential host tree occurs at the point of boring into the phloem tissues.

This finding further supports the theory of Callahan (1952 in Wood 1972) - that random landing of beetles precedes the discrimination between trees, which in turn occurs prior to sustained feeding. The subsequent response of dispersing beetles to the beetles initiating galleries is influenced by specific pheromones produced by the sex initiating the gallery. However, Ips paraconfusus (Wood et al, 1966) and D. pseudotsugae (Jantz and Rudinsky, 1965) produce their aggregating pheromones even when they are forced to feed on non-host species. But sustained feeding only occurred on suitable food plants (Wood and Bushing, 1963).

Currently it is known that both biosynthesis and release of pheromones are triggered by different mechanisms in different bark beetle species, depending on their aggressiveness (Vité et al, 1972). Mere exposure to oleoresin may result in the production of pheromones in certain bark beetle species (Hughes, 1973 a,b; Vité et al, 1972), extensive feeding ultimately terminating this biosynthesis (Coster and Vité, 1972). Such pheromones are termed 'contact type', in contrast to 'frass type' pheromones that are produced only after sustained feeding. Those species producing the 'frass type' of pheromone cannot aggregate before the

susceptibility of the host has been determined. Such a species is I. grandicollis which needs to feed on suitable host material for the production of the aggregation pheromone, ipsenol (Vité et al, 1972; Hughes, 1974).

The study on the mating behaviour of I. grandicollis ruled out earlier speculations on the habits of this species and clearly defines the effect of the micro-environment upon it. Mating among mature adults, including those adults that have not yet emerged from the host in which they developed, always takes place within galleries in the bark, with associations outside the bark, not resulting in any mating.

The ability of females, both virgin and mated (those mated prior to their first emergence and those that have already oviposited) to initiate galleries has been already recognised (Wilkinson, 1964; Morgan, 1967; All and Anderson, 1972).

Extension of these results during this study clearly demonstrates that males will enter most galleries initiated by females. Moreover, not only are virgin females accepted into nuptial chambers by resident males, but also mated females as well are accepted, again resulting in the commonly observed sex ratio in breeding attacks.

The amount of pheromone in frass or gut extracts have hitherto been expressed either as GC/GLC peak heights (Hughes, 1974; Hughes and Renwick, 1977) or by comparison of GLC peak areas with known amounts of the synthetic compounds (Byers et al, 1979).

A more reliable method of expressing such concentrations is to incorporate an internal standard of known concentration into extracts. Measurement of the ratio of GC/MS peak areas of single ions of the internal standard and of the pheromone and comparison with a standard curve of this ratio obtained with known concentration of the synthetic pheromone provide a sensitive and precise measurement of the pheromone concentration. Also the amount of ipsenol contained in male beetles is expressed per body weight (g) of the insect in order to permit comparison with other species of bark beetles.

Studies on the conditions under which the aggregating pheromone is produced in I. grandicollis related well with the known behaviour pattern of this and related species. Experiments of Werner (1972a) with virgin male I. grandicollis showed that frass of maximum attractiveness was produced on the second and third days of boring into pine bolts. Wood et al (1966) found that in Ips paraconfusus frass of maximum attractiveness was produced after 18 hr of boring.

The present study indicates that the amount of ipsenol within individual males of I. grandicollis reaches a peak after they have been boring for 24 hr, generally confirming data of Wood et al (1966) and not differing greatly from that of Werner (1972a). Any discrepancy between the respective data may be due to the differences in the method by which the pheromone was extracted. The amount of ipsenol contained in mated males did not differ significantly from that of unmated males, confirming the findings of Vité and Gara (1962); Gara (1963) and Vité and Pitman (1969), but

contradicting those of Ward (1967) and Werner (1972b).

Although the pheromone is contained in the faecal pellets, in previous studies ipsenol was extracted from frass collected over a cumulative period, while in the present study ipsenol was extracted from material retained in the gut at a particular time in the feeding sequence.

The initial detection of ipsenol in extracts of I. grandicollis (after 9 to 12 hr of boring), and the appearance of faecal pellets in frass at about the same time, is in agreement with the concept of the production of the frass-type of pheromones following feeding (Vité et al, 1972). It further emphasises the importance of the initial boring activity prior to sustained feeding, necessary to establish the susceptibility of the host for successful colonisation, and provides evidence for previous theories on the necessity of an ingested precursor for the production of the pheromone (Hughes, 1974).

The absence or non-production of the pheromone in adult males of I. grandicollis that had bored into 'resistant' host trees, indicates that insects during such test-bores do not feed, thereby ensuring the non-production of the pheromone in unsuitable ('resistant') host trees. However, the possibility that beetles during their test-bores retreat on reaching the inner bark which is usually the source of food of bark beetles, also remains as another possibility.

Finally, this study emphasises the fact that basic progress in bark beetle control is only likely to be achieved through a knowledge of the insect and its host. Although much is known about

the scolytids inhabiting conifers, detailed research on each species' habits specifically during host selection, gallery initiation, pheromone production and mating, is important. A thorough understanding of their biologies and their behavioural processes will lead to a better assessment of those factors, important to the economic destructiveness of the species and to their manipulation for effective control measures.

However, the accuracy of any prediction of outbreaks, remains subjective due to the influence of the environment on the interaction of the insect and its host.

APPENDICES

Appendix 3.1Analysis of variance for sap potential (S.P) of P. radiata trees

SV	DF	SS	MS	F	P
Treatments :	(146)	(1329.13)	(4.29)		
Times	2	87.22	43.61	10.17	>0.01
Trees	48	737.05	15.36	3.58	>0.01
Interaction	96	504.87	5.26	1.22	
Error	147	630.38			
Total	293	1959.50			

Appendix 3.2

Analysis of variance for oleoresin exudation pressure (O.E.P)
of P. radiata trees

SV	DF	SS	MS	F	P
Treatments :	(146)	(431.63)			
Times	2	9.7	4.85	21.31	>0.01
Trees	48	397.94	8.29	36.45	>0.01
Interaction	96	23.99	0.25	1.09	
Error	441	100.32	0.23		
Total	587	531.94			

Appendix 3.3

Attack scores for each of the treatments and their replicates
 et each of the cage sites.

(3 observations/cage site)

Treatments	Replicates			Cage sites
	1	2	3	
BG	3,4,5	2,1,2	0,1,1	(1)
	3,4,2	3,4,3	3,3,3	(2)
	2,1,0	3,4,1	4,2,4	(3)
CG	3,3,2	2,1,0	3,4,3	
	2,1,3	2,2,2	0,1,0	
	3,4,1	3,4,2	4,3,2	
LI	4,3,4	4,3,4	3,2,3	
	0,1,0	2,3,2	2,1,0	
	3,0,4	2,0,1	2,0,2	
F	5,5,5	5,5,5	5,5,5	
	5,5,5	5,5,5	5,5,5	
	5,5,5	5,5,5	5,5,5	

Appendix 3.4

Analysis of variance for soil moisture content at sites adjacent to test
P. radiata trees.

SV	DF	SS	MS	F	P
Sites	6	5750.17	958.36	0.73	< 0.05
Depths	2	13744.59	6872.29	5.22	> 0.05
Error	12	15796.90	1316.4		
Total	20	35291.67	1764.58		

Appendix 3.5

Effect of various treatments on P. radiata O.E.P., S.P. levels and their effect on the number of galleries initiated by I. grandicollis.

(No. of replicates/treatment = 3) (No. of cage sites/ tree = 3).

Tree category	Treatment	OEP in bars		SP in bars		No. of attacks per tree
		Before	After	Before	After	
Trees with high OEP and SP levels	Base	4.6	3.0	28.0	13.1	6.2
	Girdled (B.G.)	3.8	2.8	26.5	14.0	5.3
		4.3	3.1	24.0	14.0	3.5
	Crown	3.9	3.6	27.0	12.3	5.6
	Girdled (C.G.)	3.8	3.0	27.3	14.6	4.2
		4.2	3.8	26.33	15.6	3.7
	Left	3.9	4.1	28.0	18.6	3.7
	Intact	4.4	4.4	26.7	18.3	5.2
	(I)	4.3	4.1	26.7	18.9	3.6
	Felled	4.4	0	26.7	0	25.8
	(F)	3.9	0	25.2	0	23.4
		3.9	0	25.3	0	27.4
Trees with low OEP and SP levels	Base	2.3	1.4	21.5	11.3	4.5
	Girdled (B.G.)	2.4	1.5	21.5	13.3	5.9
		2.3	1.1	22.2	13.6	3.8
	Crown	2.6	2.0	20.7	13.8	6.1
	Girdled (C.G.)	2.36	1.9	21.7	19.7	5.8
		1.8	1.4	21.7	13.2	4.6
	Left	1.9	1.9	21.3	16.3	4.4
	Intact	2.1	2.3	22.2	16.0	6.8
	(I)	1.6	1.8	22.3	17.3	4.6
	Felled	2.5	0	22.8	0	30.2
	(F)	2.2	0	20.8	0	24.2
		2.3	0	23.0	0	28.7

Appendix 3.6

Analysis of variance for the number of attacks/tree made by caged
I. grandicollis adults, on the two categories of trees subjected to
 3-treatments (C.G., B.G., L.I.)

SV	DF	SS	MS	F	P
Bt. treatments	5	3.66	0.73	1.11	>0.05
Bt. replicates	2	7.81	3.90	5.90	>0.05
Error	10	6.61	0.66		
Total	17	18.08	1.06		

Appendix 5.1

Analysis of variance of the responses of the field and caged

I. grandicollis populations to the various treatments.

Response	Population	SV	DF	SS	MS	F	P	
Male initiated galleries	Field	Treatment	5	5647.87	1129.57	22.19	0.01	
		Error	24	1221.60	50.9			
		Total	29	6869.47	236.88			

	Lab	Treatment	5	2497.87	493.57	63.69	0.01	
		Error	24	186.00	7.75			
Total		29	2683.87	91.51				

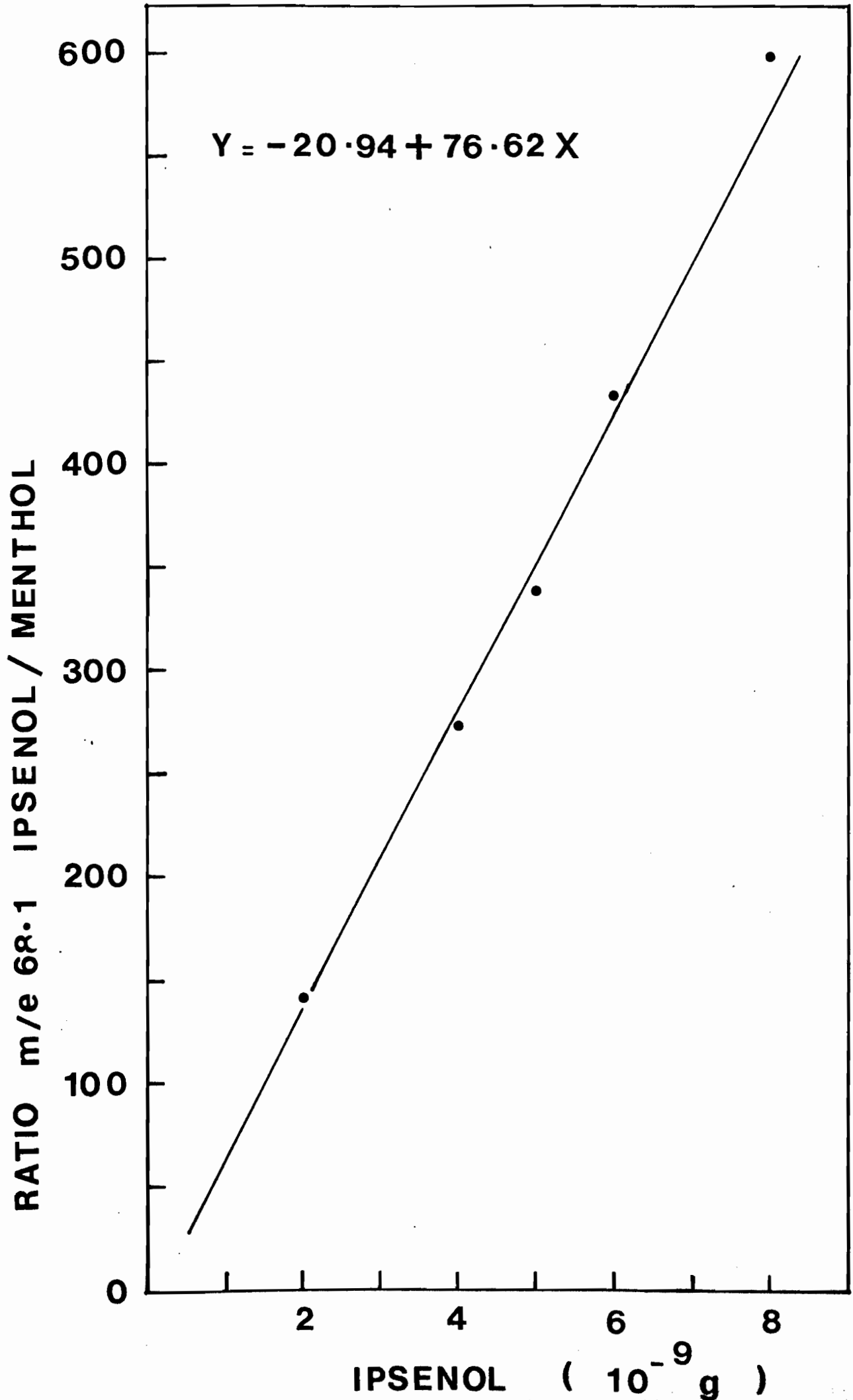
Females joining male galleries	Field	Treatment	5	21989.1	4397.82	9.34	0.01	
		Error	24	11303.2	470.97			
		Total	29	33292.3	1148.01			

	Lab	Treatment	5	2856.7	571.34	13.52	0.01	
		Error	24	1014.0	42.25			
Total		29	3870.7	133.47				

Female initiated galleries	Field	Treatment	5	19.9	3.98	6.45	0.01	
		Error	24	14.8	0.62			
		Total	29	34.7	1.19			

	Lab	Treatment			8.97	1.17	2.83	0.01
		Error			15.20	0.63		
Total				24.17	0.83			

Appendix 6.1 Standard curve showing the relationship
between the ratio of area of m/e 68.1 peak
of ipsenol (R.T. 2.4 min) to menthol (R.T.
4.2 min) with varying concentrations of
ipsenol.



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Note: Several important
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