STUDIES ON INSECT DISSEMINATION

OF WOOD ROTTING FUNGI

A Thesis

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By

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TABLE OF CONTENTS

INTRODUCTION

The wood rotting fungi which form perennial sporocarps such as Fomes spp. are known for their ability to produce large numbers of basidiospores. It has been calculated that a single large sporocarp of Fomes applanatus (Pers. ex Wallr.) Gill. can release 30 billion spores every day throughout the growing season (Buller, 1922). It is not surprising then, that the air is often heavily contaminated with these spores, and that they may be carried great distances by the wind (Stakman and Christensen, 1946). This is consistent with the general acceptance of the idea that wood rotting fungi are wind disseminated, although there is little direct evidence to show that their spores readily survive the air-borne journey and can become successfully established upon arrival (French and Christensen, unpublished).

It is also well known that a wide variety of insects and other arthropods commonly visit or attack the sporocarps of these fungi. Basidiospores must certainly adhere to many of these insects. Thus, the possibility exists that the contaminated insects carry spores to suitable inoculation sites.

The importance of insects as disseminators of many other fungi has long been recognized and has been the object of a great deal of research (Spaulding, 1903 and Leach, 1940). Insects are ideally suited for the transmission of fungi because of their hairyness and mobility (Spaulding, 1903). Dry spores as well as those in sticky matrices adhere well to insects and the spores of many fungi can

pass through the insect gut unharmed (Leach, 1940). Insects are also more likely to take the inoculum directly to a favorable site for fungus growth than the wind, and the wind may deposit spores on insects reducing the need for direct sporocarp-insect contact. In spite of this, insect dissemination of wood rotting fungi, as an alternative to wind dissemination, has not been seriously investigated.

Insects associated with the sporocarps of wood decay fungi can be loosely divided into two groups. One group consists of those insects which randomly or accidentally land on a sporocarp or are attracted to it very casually as they might be attracted to many other objects; many Diptera fall into this category. The second group are those insects which are specifically attracted to a sporocarp for particular food needs or as a breeding site. Within the second group exists every gradation of insect-fungus association, from a very loose association such as that between nitidulid beetles and the oak wilt fungus to a highly specialized, mutualistic relationship such as that between ambrosia beetles and their fungi. In general the fungi in some way benefit the insects in the second group, whereas this is not necessarily the case for the insects in the first group.

The insects of most interest in the second group would be those whose normal habits or movements involve a move (or repeated moves) from the sporocarp of a wood rotting fungus to a suitable inoculation site. Such insects would then be prime suspects as disseminators of that fungus.

Because the two groups of insects were investigated separate-
ly this paper will be divided into two main parts. The first part
describes the experiments on dissemination of wood rotting fungi
by the first group, or what will be called "random" dissemination.
The second part is an investigation of the habits and movements of
the tenebrionid beetle Bolitotherus cornutus (Panz.) which is
closely associated with F. applanatus. This study was made to
determine whether the movements of this beetle fit the pattern of
a good vector described above. This type of dissemination will be
called "direct" dissemination.

The purpose of this investigation was to determine whether
insect dissemination of wood decay fungi does occur by either or
both of the random and direct means. However, the failure to
develop a reliable technique for isolating wood rotting fungi
from insects and the fact that wood rotting fungi naturally grow
slowly in wood has limited the conclusions which can be drawn,
especially from the experiments on random dissemination.

Leach, in his book "Insect Transmission of Plant Diseases",
does not cite a single documented example of insect dissemination
of a wood rotting fungus. In contrast to this, the literature on
insect dissemination·of other tree attacking fungi such as blue
stain and ambrosia fungi is very extensive (see the literature
reviews by Franche-Grosmann, 1963, Baker, 1963, and Parkin, 1942).

Although these insect-fungi relationships are outside the
scope of this paper, a possible link between ambrosia fungi and
wood rotting fungi was found when Bakshi (1952) cultured the

ambrosia fungus Oedocephalum lineatum from the galleries of
Trypodendron lineatum (spruce ambrosia beetle). The fungus was
similar in culture to Fomes annosus and would grow in and destroy
scotch pine blocks. He concluded that the ambrosia fungus was the
conidial stage of the wood rotter F. annosus, and that it was being
transported by the beetle. However, to my knowledge, this has not
been followed up.

Talbot (1952) is the only person to experiment directly with
the insect dissemination of wood decay fungi. He showed that a
variety of invertebrate inhabitants of forest litter and rotting
logs (wood lice, mites, centipedes, slugs, springtails, and worms)
were capable of picking up and transporting the spores of a variety
of fungi. Most of the fungi he isolated and identified from these
organisms were Hyphomycetes, but many unidentified Basidiomycete
spores were also obtained. The feces of wood lice in particular
were shown to contain viable Basidiomycete spores.

All other reports of wood decay fungi and insect association
in which insects are implicated as disseminators have emerged
mainly from studies on the deterioration of fire or insect damaged
forest stands.

Basham and Belyea (1960) isolated an unknown Hyphomycete
(Fungus F), which was rotting the sapwood of dead balsam fir, from
the mouth parts, legs, abdomen, and eggs of the sawyer beetle,
Monochamus scutellatus. They concluded that the beetle was carry-
ing the fungus, and that it could be inoculating the trees either
during the chewing of the egg notch by the adult or during ovi-
position.

Other insects, while not actually disseminating wood rotting
fungi have been shown to be important factors in allowing the fun-
gi to gain entrance to wood. Leach, Orr, and Christensen (1937)
and Basham and Belyea (1960) found the wood rotters _Peniophora
gigantea_ and _Polyporus abietinus_ associated with _Monochamus_ larval
tunnels in red pine and balsam fir, respectively. Smerlis (1957)
and Whitney (1961) showed the wounds made by _Hylobius_ were associ-
ated with wood decay in balsam fir and spruce, respectively, and
that these wounds were more important than wounds made by other
agents. Whitney (1952) showed the same thing for _Hypomolyx_
(spruce root borer) in white spruce, and Ostrander and Foster
(1957) found weevil wounds associated with _Fomes pini_ in eastern
white pine.

While the literature dealing directly with insect dissemina-
tion of wood rotters is scanty, a number of papers have been pub-
lished listing the insects associated with bracket fungi. Some
of these papers include ecological notes on the insects. (Wiess,
1920 a, b, 1921, 1923, Wiess and West, 1920a, 1921, Scheerpeltz
and Höffer, 1948, Kessel and Kessel, 1939a, b, and Graves, 1960).
Thus, the insect fauna of certain fungi such as _Polyporus
versicolor_, _P. sulphureus_, _P. lucidus_, _Fomes applanatus_, _F.
fomentarius_, _Pleurotus ostreatus_, etc., is relatively well known,
but no mention was found in any of these papers of possible dis-
semination of the fungi by the insects.

One wood rotting fungus, _Polyporus volvatus_ seems, by the
construction of the sporocarp, to be designed for insect

dissemination. The pore surface is covered with a tough membrane which develops an opening when the fruit body is mature. Insects are able to enter the spore-filled chamber where they become covered with spores. Hubbard (1892) described and listed the insects commonly found in the fungus and stated, "and these visitors, it may readily be believed, play an important part in the dissemination of the spores and the propagation of this fungus." However, he offers no experimental proof for this statement.

Literature concerning some of the techniques used will be mentioned where pertinent and the literature on Bolithotherus cornutus will be discussed in the second section.

SECTION I.   STUDIES ON RANDOM, INSECT DISSEMINATION

OF WOOD ROTTING FUNGI

Description of the Study Areas

The field work was carried out near the University of
Minnesota Biological Station in Itasca State Park during the
summers of 1964 and 1965. Several study areas, all located in
hardwood stands, were used and are described as follows:

Site I: located just south of the Biological Station, was
a predominately maple-basswood climax stand which had nearly re-
placed the overmature aspen and large red pines in the area. The
area contained several large aspen logs bearing numerous sporo-
carps of Fomes applanatus which supported large populations of
Bolitotherus cornutus. Thus, the area was used for most of the
work on the behavior of this beetle and a detailed description
of the study area will be given in the second part of this paper.

The following two aspen stands were used for experiments with
random dissemination:

Site II: was a small stand of overmature aspen mixed with
spruce immediately east of the Biological Station. Approximately
half of the aspen trees in this stand bore active sporocarps of
Fomes igniarius (L. ex Fries) Kickx. Probably most of the aspen
without sporocarps were also infected with heart rot. (Schmitz
and Jackson, 1927 and Riley, 1952).

Site III: was a stand of young aspen between twenty and
twenty-five years old, about 350 yards northwest of Site II.

As well as could be determined, the stand was free from any type of wood decay fungi.

Site IV: was a small clearing in an open stand of large red pine mixed with birch about 500 yards west of Site II. The area was chosen for its openness and lack of sporocarps of any _Fomes_ spp., and was used for a dispersal experiment with _B. cornutus._

Fomes applanatus and _F. igniarius_ were used in this study because the sporocarps of both species were very common. Like all _Fomes_ spp. they produce perennial sporocarps which add a new spore producing layer or hymenium each year.

Fomes applanatus is a common and familiar fungus found growing on logs and dead stumps of many hardwoods but mainly on aspen and birch in the Itasca area. The sporocarp is sessile, applanate, and usually in the shape of a semicircular shelf which projects out $1\frac{1}{2}$ to 12 inches and is $1\frac{1}{2}$ to 16 inches wide. The surface is gray, the context is dark brown and woody, and the hymenial surface is white (Overholts, 1953). The spores are brown and on humid days they can be seen issuing from the hymenium and drifting away like a thin stream of brown smoke. The spores often cover the surface of the sporocarps unwashed by rain giving them a rusty brown color.

Fomes igniarius is also sessile, but on aspen it is more ungulate than _F. applanatus._ The sporocarps are $1\frac{1}{2}$ to 8 inches wide and they project out $\frac{1}{2}$ to 6 inches. The surface is gray to black, the context dark brown, woody, and very hard. The hymenial surface is at first grayish tan but later brown. This species is

common on mature living aspen in the Itasca area but is found on other hardwoods as well (Overholts, 1953).

Outline of the Procedure

Merely a brief description of the procedures will be mentioned here, with the details of the methods and techniques used discussed where appropriate.

Three indirect experiments were to determine whether random dissemination does occur. In Site II, insects were trapped which were attracted to both the Fomes igniarius sporocarps and to artificially made wounds on aspen in the stand. Wound traps were also established in Site III, an area with no sporocarps, to provide a comparison with the insects trapped in Site II. The insects trapped in all three sets of traps (plus a set of controls) were compared to determine whether spore covered insects were likely to land on exposed wounds during their life span. The second experiment was an effort to isolate wood rotting fungi from a sample of the insects collected at both the sporocarp and wound traps to determine if insects from both types of traps were carrying spores.

In the third experiment, insects and other arthropods were contaminated with the fungus Hormodendrum resinae (Lindau) and subsequently allowed to come in contact with artificial wounds. The fungus was reisolated from the wounds to show that the insects commonly collected in the traps in Site II and III could carry and deposit spores on a wound surface. Hormodendrum resinae is a unique fungus species in its ability to grow on creosote agar,

thus eliminating the problem of contaminates.

The same technique was used to test for the transmission and inoculation of a wood rotter by an insect by using the beetle B. cornutus contaminated with F. applanatus spores.

The study of the habits and movements of B. cornutus in Site I was carried out by day and night observations and by recording the movement of beetles individually marked with a non-toxic paint.

In site I and IV sporocarps were introduced around isolated populations of marked beetles to test the ability of the beetles to locate and colonize new sporocarps. The time lapsed before individual beetles moved to introduced sporocarps from the central population was recorded.

Wound and Sporocarp Trap Experiments

Random dissemination of wood rotting fungi was investigated indirectly by establishing wound and sporocarp traps to determine whether the same insect groups would regularly appear in both.

A set of sporocarp traps, wound traps, and control traps were established in Site II, plus an additional set of wound traps in Site III, making a total of four sets of traps. Thus, the insects collected at wound and sporocarp traps in a stand containing a large amount of F. igniarius could be compared to insects collected from wounds in a stand free from F. igniarius and any other wood decay. It is assumed that, if a large number of arthropods of a particular group (family or order) appear at both the sporocarp and wound traps, it is highly likely that individuals would have

visited both some time during their life span. If this does occur, random dissemination would be a distinct possibility.

Methods

In Site II, the _F. igniarius_ sporocarps were usually located from 5 to 30 or more feet above ground on the bole of the aspen. Only sporocarps which could be reached from the ground (5 to 6½ feet high) were selected for traps. To trap the insects attracted to the sporocarps a tree banding compound, Acme Insect Stop (Acme Quality Paints Incorporated, Detroit, Michigan; in 1965 Tree Tanglefood, Tree Tanglefoot Company, Grand Rapids, Michigan was used), was spread on the bark around the sporocarp in a band between 2 and 3 inches wide. Insect Stop was also applied to the top surface of the sporocarps leaving only the pore surface free from the compound (Fig. 1). A standard 1 inch paint brush was used for the application.

Insects landing on or near the fungus became entangled in the compound. Several sporocarp traps were established on some individual trees but each "painted" sporocarp was counted as a separate trap. The dates the eight sporocarp traps on four trees and the 16 sporocarp traps on seven trees were established in 1964 and 1965, respectively, are shown in table 1.

Wound traps in Site II and Site III were established about 5 feet from the ground except for four (two in each site) added in 1965 which were 1 foot from the ground. In Site II aspen were chosen which bore no sporocarps and were more or less evenly spaced throughout the stand. The distance from trees selected

for wound traps and the nearest sporocarp trap varied between 15

and 50 feet.

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Figure 1. Sporocarp trap, site II.

Figure 2. Wound trap, site II.

wounds in Site III so a comparison could be made of the effective-

ness of the new traps.

for wound traps and the nearest sporocarp trap varied between 12

and 50 feet. In Site III, trees were selected along the edge of

the young aspen stand. Wounds were made with a brick-layer's

hammer using the chisel-like end to chop a hole about 2 inches

square through the bark and into the sapwood about $\frac{1}{2}$ inch. Some

wounds had an angled bottom edge (Fig. 2), or were triangular in

shape. Only one wound was made on each tree. The dates the eight

wound traps were established in Sites II and III for both 1964 and

1965 are shown in Table 1, plus the date two additional traps were

established in Site III in 1965.

Originally the wounds were covered with $\frac{1}{2}$ gallon, cardboard

ice cream cartons. The tops of the cylindrical cartons were

fitted with 50 mesh copper screen funnels with a $\frac{1}{2}$ inch hole.

The bottoms were removed and the sides cut to fit the contours

of the individual trees and their bark. The inside walls were

smeared with Insect Stop. The cartons were placed over the wound

and held in place with twine. It was assumed that insects attracted

to the wound would enter the carton, land eventually on the walls,

and become caught in the Insect Stop.

In practice the carton traps were unsuccessful. Insects were

observed landing on the carton, but very few were caught. For this

reason the cartons were removed in Site II after they had been in

place 11 days. Insect Stop was then applied in a 3 inch border on

the bark around the wound. Carton traps were left in place on four

wounds in Site III so a comparison could be made of the effective-

ness of the new traps.

Three control traps were set up in Site II in 1964 and 1965. These consisted simply of a circle of Insect Stop with an outside diameter of 7 to 8 inches painted on an aspen bearing no sporo- carps or wounds (Fig. 3).

All the traps were checked for insects and other arthropods every two days in 1964 unless rain forced postponement of the col- lection one day. The insects were removed and put in dry vials labeled with the tree number. The insects removed from the sporo- carp traps on the same tree were labeled only as collected from that tree. Most of the insects were determined to family, but those in very poor condition and the other arthropods were deter- mined to order. The groups collected plus the number of individ- uals collected in each group was recorded for each trap.

In 1965, five trees bearing traps from the previous year had been cut down in Site II due to the construction of new sewer lines. Those that remained plus the wound traps in Site III were "repainted" with Insect Stop, and some new traps were established (Table 1). In 1965 the traps were checked and the insects removed once a week rather than every 2 days as in 1964.

Method of Analysis

To indirectly show that random dissemination occurs, a rela- tively large number of insects in a particular group should be regularly caught in both the sporocarp and wound traps. Thus, the data have been first analyzed quantitatively to determine the num- ber of times the insect and arthropod groups (plus the number of individuals in these groups) appeared in each of the four sets of

Table 1.   Dates established and number of specimens on upper sticky
for trapping insects in 1964 and 1965.   (So w specimens.)

1964

Sycamore Trees Site II

No.   Date estab.   No. of Bee   So attack   No.   Date estab.   No.
of Bee

1   7-13   1   1
2   7-13   2
3   7-13   1
4   8- 3   2

No.   D

1
2
3
4
5
6
7
8

*Nos. 1-4 originally estab.
with carton traps

Figure 3.   Control trap, site II.

Mount Trees Site III

No.   Date estab.   No.   Date estab.

1-4   8-14**   1-4   6-17
4-5   8-15   4-6   6-17
                                                              7-26

**Cartons left in place in 1964   10   7-26

Control Traps Site II

No.   Date estab.   No.   Date estab.

1-3   8-3   1,2   6-17
                                                              Cut down
                                                         3   7-26

Table 1. Dates established and number of sporocarps on aspen used for trapping insects in 1964 and 1965. (Sc = sporocarp)

<u>1964</u>   <u>1965</u>

Sporocarp Traps Site II

No.	Date estab.	No. of Scs	Sc added	No.	Date estab.	No. of Scs
1	7-13	1		1	cut down	
2	7-13	2	8-3	2	6-17	4
3	7-13	1	8-3	3	6-17	2
4	8- 3	2		4	6-17	2
				6	6-17	3
				7	6-17	1
				8	6-17	3
				9	6-17	1

Wound Traps Site II

No.	Date estab.	Cartons removed	No.	Date estab.
1	8-6*	8-17	1	6-17
2	8-6	8-17	2	Cut down
3	8-6	8-17	3	6-17
4	8-6	8-17	4	Cut down
5	8-17		5	6-17
6	8-17		6	6-17
7	8-17		7	6-17
8	8-17		8	Cut down
			9	6-17
			10	7-26
			11	7-26

*Nos. 1-4 originally estab.
with carton traps

Wound Traps Site III

No.	Date estab.	No.	Date estab.
1-4	8-14**	1-4	6-17
4-8	8-18	4-8	6-17
		9	7-26
		10	7-26

**Cartons left in place in 1964

Control Traps Site II

No.	Date estab.	No.	Date estab.
1-3	8-3	1,2	6-17
		3	Cut down
		3	7-26

traps. Secondly, "percentages of frequency" have been computed

to determine whether the most commonly collected groups regularly

appeared in both the sporocarp and wound traps.

The data from the two summers were compared separately because

the traps were established late and at varying times in 1964, while

in 1965 all the traps except one control trap and four wound traps

were operated from June 17 to August 25. Because of the number of

traps operated and the number of times the traps were checked varied

from one set, and from one year to another, a basis of comparison

was obtained by multiplying the number of checks times the number

of traps for each set of traps. This gave the number of "trap

checks" for each set of traps for each year (Table 2). Wound

Table 2. The number of trap-checks for each set of traps, exclud-
ing the wound traps covered with cartons.

	1964			1965		
	No. of Traps	No. of Checks	Trap-Checks	No. of Traps	No. of Checks	Trap-Checks
Sporocarp traps	4	18	128	16	10	160
Site II	4	14				
Wound traps	4	7	56	6	10	70
Site II	4	7		2	5	
Wound traps Site III	4	6	24	8	10	90
				2	5	
Control traps	3	14	42	2	10	25
Site II						

traps covered with cardboard cartons were not included in these

figures because of their failure to trap insects. Both the number

of times each group was collected and the number of individuals

collected in each group from each of the traps were divided by the
appropriate number of "trap checks". This (X100) gave the com-
puted values used in the analyses.

For the quantitative data, a value representing the frequency
with which a particular group appeared per trap per check, and a
value representing the number of individuals which appeared per
trap per check for each set of traps was obtained.

The frequency-per-trap-per-check-value for a particular group
from one set of traps over the same value from another set of
traps (X100) gave the "percent of frequency". The percentages
were computed once using the sporocarp traps and once using the
wound traps (Site II) on the basis of comparison with each of the
other three sets of traps. This was done for the seven groups
which were most commonly collected in the traps. Percentages
figured for the number of individuals collected per trap per check
showed the same relationships and are not presented.

In a third analysis reciprocal comparisons were made of the
number of insect families each set of traps had caught in common
with the other sets. The number of families from each of three
sets of traps which were also collected in the fourth set, over
the total number of families collected in the fourth set gave the
comparative values. This was done for each set of traps in turn.
Two comparisons were made; one using all the families trapped in
1965 and a second using the total number of families trapped both
years. This provided an over-all picture of the amount of similar-
ity in the insect fauna attracted to the various traps for one
summer season (1965) and also for the total number of families

collected in the two years.

Because a fairly large number of families were collected only once at each set of traps, it was thought a more valid picture of the similarity in the insect fauna between the various sets of traps would be obtained if these families were deleted from the comparisons. Therefore, the comparisons were duplicated using only the families collected two or more times.

Results

Table 3 lists all the families and orders collected in each set of traps according to whether they were collected the first or second summer only, or during both summers. Also listed are the actual and computed values for both the number of times each group was collected and the number of individuals collected in each group. Table 3 shows that the groups collected in both 1964 and 1965 were, for the most part, the groups collected in the largest numbers as well. Rhagionidae (Diptera) was the major exception. While trapped in large numbers in the second summer none were trapped in 1964 because ragionids were abundant only during the first half of the summer; a period when the traps had not yet been set up in 1964. The few arthropods collected in the wound traps covered by cardboard cartons are not listed. The families Cicadellidae, Mycetophilidae, Chironomidae, Muscidae, Rhagionidae, and the orders Lepidoptera and Araneida were the most commonly and consistently collected groups. Thus, it is assumed that these groups are the most likely potential disseminators, and accordingly are dealt with in more detail. Other groups appearing in the traps

Table 3. List of the groups caught in the four sets of traps
showing the actual and computed values for the number
of times and number of individuals trapped.

Sporocarp Traps Site II

Families, Orders Collected	No. of Times Collected 1964	1965	No. of Individ. Collected 1964	1965	% of Times Collected 1964	1965	% of Individ. Collected 1964	1965
1964 Only								
Cercopidae	4		5		3.1		3.9	
Cixiidae	1		1		.8		.8	
Derbidae	1		1		.8		.8	
Endomyciidae	1		1		.8		.8	
Cecidomyiidae	1		1		.8		.8	
Dolichopodidae	2		2		1.6		1.6	
Empididae	1		1		.8		.8	
Drosophilidae	1		1		.8		.8	
Tachinidae	1		1		.8		.8	
Ichneumonidae	2		2		1.6		1.6	
Formicidae	1		1		.8		.8	
Both 1964,5								
Cicadellidae	18	12	31	15	14.0	7.5	24.2	9.4
Limnephilidae	1	4	1	4	.8	2.5	.8	2.5
Elateridae	1	3	1	6	.8	1.9	.8	3.8
Lampyridae	2	3	2	4	1.6	1.9	1.6	2.5
Mycetophilidae	4	3	4	3	3.1	1.9	3.1	1.9
Chironomidae	2	5	3	5	1.6	3.1	2.3	3.1
Anthomyiidae	1	1	1	1	.8	.6	.8	.6
Muscidae	12	7	14	9	9.4	4.4	10.9	5.6
Araneida	2	4	4	4	1.6	2.5	3.1	2.5
Phalangida	1	4	1	5	.8	2.5	.8	3.1
Corrodentia	3	1	3	1	2.3	.6	2.3	.6
Lepidoptera	1	10	1	12	.8	6.2	.8	7.5
Diptera	1	8	1	9	.8	5.0	.8	5.6
1965 only								
Leptoceridae		5		6		3.1		3.8
Melandryidae		2		2		1.3		1.3
Buprestidae		1		1		.6		.6
Tipulidae		4		4		2.5		2.5
Rhagionidae		8		18		5.0		11.3
Tenthridinidae		1		1		.6		.6
Trichoptera		3		3		1.9		1.9
Coleoptera		2		2		1.3		1.3

Table 3. Continued

Wound Traps Site II

Families, Orders Collected	No. of Times Collected		No. of Individ. Collected		% of Times Collected		% of Individ. Collected	
	1964	1965	1964	1965	1964	1965	1964	1965
1964 only								
Psocidae	1		1		1.8		1.8	
Cixiidae	1		1		1.8		1.8	
Chaoboridae	1		1		1.8		1.8	
Phoridae	2		2		3.6		3.6	
Sarcophagidae	2		3		3.6		5.4	
Both 1964,5								
Cicadellidae	10	11	15	11	17.9	15.7	26.8	15.9
Cercopidae	1	1	1	1	1.8	1.4	1.8	1.4
Chironomidae	7	2	7	2	12.5	2.9	12.5	2.9
Mycetophilidae	6	4	6	4	10.7	5.7	10.7	5.7
Muscidae	4	8	4	10	7.1	11.4	7.1	14.3
Araneida	4	5	5	6	7.1	7.1	3.9	8.6
Corrodentia	1	1	1	1	1.8	1.4	1.8	1.4
Lepidoptera	1*	5	1*	6	-	7.1	-	8.6
Diptera	2	8	2	12	3.6	11.4	3.6	17.1
1965 only								
Miridae		1		1		1.4		1.4
Aphididae		1		1		1.4		1.4
Panorpidae		1		1		1.4		1.4
Limnephilidae		1		3		1.4		4.3
Leptoceridae		2		3		2.9		4.3
Amatidae		1		1		1.4		1.4
Lycidae		2		2		2.9		2.9
Oedemeridae		2		2		2.9		2.9
Cleridae		1		1		1.4		1.4
Buprestidae		1		1		1.4		1.4
Empididae		2		2		2.9		2.9
Tachinidae		1		1		1.4		1.4
Ichneumonidae		2		2		2.9		2.9
Chalcidoidea		1		1		1.4		1.4
Rhagionidae		15		55		21.2		78.6
Phalangida		6		7		8.6		10.0
Trichoptera		4		4		5.7		5.7
Coleoptera		1		1		1.4		1.4

*Larva

Table 3. Continued

Wound Traps Site III

Families, Orders Collected	No. of Times Collected 1964	1965	No. of Individ. Collected 1964	1965	% of Times Collected 1964	1965	% of Individ. Collected 1964	1965
1964 only								
Psocidae	1		1		4.2		4.2	
Cercopidae	1		4		4.2		16.7	
Hemerobiidae	1		1		4.2		4.2	
Crysopidae	1		1		4.2		4.2	
Lauxaniidae	1		1		4.2		4.2	
Endomychidae	1		1		4.2		4.2	
Dolichopodidae	1		1		4.2		4.2	
Phoridae	1		1		4.2		4.2	
Ichneumonidae	1		1		4.2		4.2	
Both 1964,5								
Cicadellidae	14	35	51	56	58.3	38.9	212.5	62.3
Lampyridae	1	2	1	2	4.2	2.2	4.2	2.2
Chironomidae	3	1	5	1	12.5	1.1	20.8	1.1
Myceotphilidae	2	2	2	2	8.3	2.2	8.3	2.2
Muscidae	8	20	12	75	33.3	22.1	50.0	83.3
Anthomyiidae	1	1	1	1	4.2	1.1	4.2	1.1
Tachinidae	1	2	1	3	4.2	2.2	4.2	3.3
Araneida	1	8	1	9	4.2	8.9	4.2	10.0
Lepidoptera	1	12	2	13	4.2	13.3	8.3	14.5
Diptera	2	15	2	20	8.3	16.7	8.3	22.1
1965 only								
Membracidae		2		2		2.2		2.2
Cixiidae		3		3		3.3		3.3
Achilidae		1		1		1.1		1.1
Ostomidae		1		1		1.1		1.1
Cantheridae		2		3		2.2		3.3
Melandryidae		1		1		1.1		1.1
Pyrochoridae		1		1		1.1		1.1
Buprestidae		1		1		1.1		1.1
Scolytidae		1		1		1.1		1.1
Tipulidae		1		1		1.1		1.1
Rhagionidae		27		141		30.0		156.8
Syrphidae		1		1		1.1		1.1
Sarcophagidae		1		1		1.1		1.1
Caliphoridae		2		3		2.2		3.3
Tenthridinidae		2		2		2.2		2.2
Formicidae		2		2		2.2		2.2
Phalangidae		1		1		1.1		1.1
Trichoptera		4		5		4.4		5.6
Coleoptera		2		2		2.2		2.2

Table 3. Continued

Control Traps Site II

Families, Orders Collected	No. of Times Collected 1964	1965	No. of Individ. Collected 1964	1965	% of Times Collected 1964	1965	% of Individ. Collected 1964	1965
1964 only								
Cercopidae	1		2		2.4		4.8	
Cixiidae	1		1		2.4		2.4	
Mycetophylidae	1		1		2.4		2.4	
Dolichopodidae	1		1		2.4		2.4	
Pompilidae	2		3		4.8		7.1'	
Tenthridinidae	1*		1*		-		-	
Ichneumonidae	1		1		2.4		2.4	
Corrodentia	4		4		9.5		9.5	
Both 1964,5								
Cicadellidae	2	4	2	7	4.8	16.0	4.8	28.0
Muscidae	5	6	5	10	11.9	24.0	11.9	40.0
Araneida	2	1	2	1	4.8	4.0	4.8	4.0
Lepidoptera	1	4	1	4	2.4	16.0	2.4	16.0
Phalangida	1	1	1	1	2.4	4.0	2.4	4.0
1965 only								
Miridae		1		1		4.0		4.0
Limnephilidae		1		1		4.0		4.0
Elateridae		1		1		4.0		4.0
Mordellidae		1		1		4.0		4.0
Melandryidae		1		1		4.0		4.0
Anobiidae		1		1		4.0		4.0
Curculionidae		1		1		4.0		4.0
Xylophagidae		1		2		4.0		8.0
Rhagionodae		4		6		16.7		24.0
Tachinidae		1		1		4.0		4.0
Diptera		5		12		20.1		47.0

*Larva

(Table 3) were not analyzed further individually because they were either collected in small numbers in the various sets of traps, or they appeared in large numbers in only one set of traps.

The relative number of times the seven common groups were collected in each set of traps is shown in Figure 4 and Figure 5 for 1964 and 1965, respectively. Figures 6 and 7 show the same for the relative number of individuals collected in each set of traps. The results from the two summers were similar except that the frequency of insects caught in the control traps was consistently higher in 1965 than in 1964. This may be an artifact caused by the fact that there was a relatively small number of control "trap checks" in 1965. This has the effect of giving even a few insects caught in the control traps a high value when figured on the per trap or per check basis. Among the seven commonly collected groups, the Cicadellidae, Muscidae, and in 1965 the Rahgionidae stand out as the most prevalent groups. However, the Muscidae appeared in the control traps at a greater frequency than in either the sporocarp or wound traps in Site II in both years.

Figures 8 and 9 show the "percentages of frequency" for the Cicadellidae, Muscidae, Mycetophilidae, Chironomidae, and Araneida for 1964 and 1965, respectively. Values were not figured for the Rhagionidae and Lepidoptera because both groups were obviously (Figs. 4 and 5) collected relatively few times in the sporocarp traps. Seventy-five per cent was arbitrarily chosen as the line between a high and a low level of common appearance of a group in two sets of traps. A per cent of 75 or higher for a particular

Figure 4. Per cent of times the common groups were collected
per trap per check in each set of traps in 1964.
(Sc=Sporocarp traps; W2=Wound traps, site II; W3=
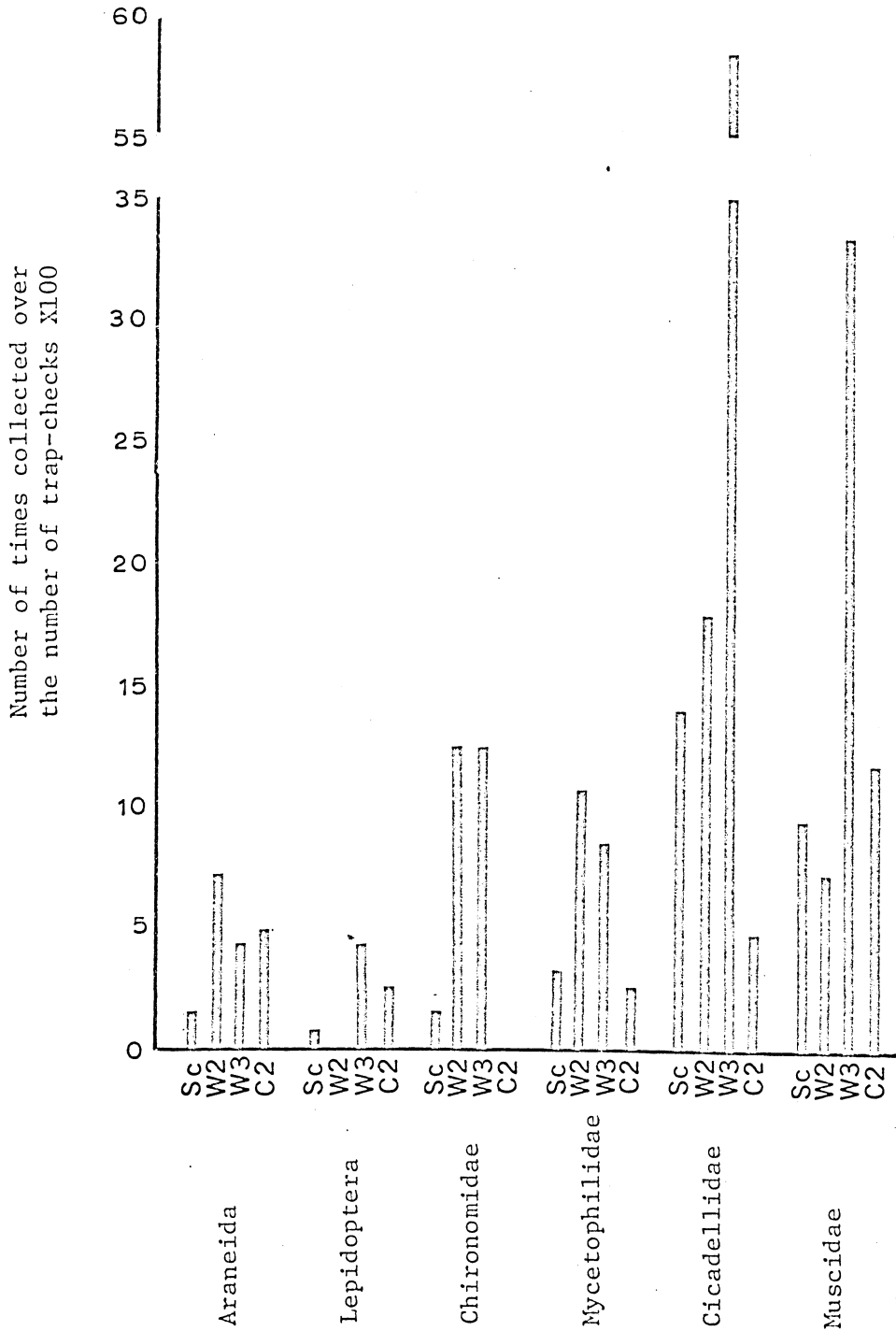Wound traps, site III; C2=Control traps, site II).

Figure 5. Per cent of times common groups were collected
per trap per check in each set of traps in 1965.
(Sc= Sporocarp traps; W2=Wound traps, site II; W3=
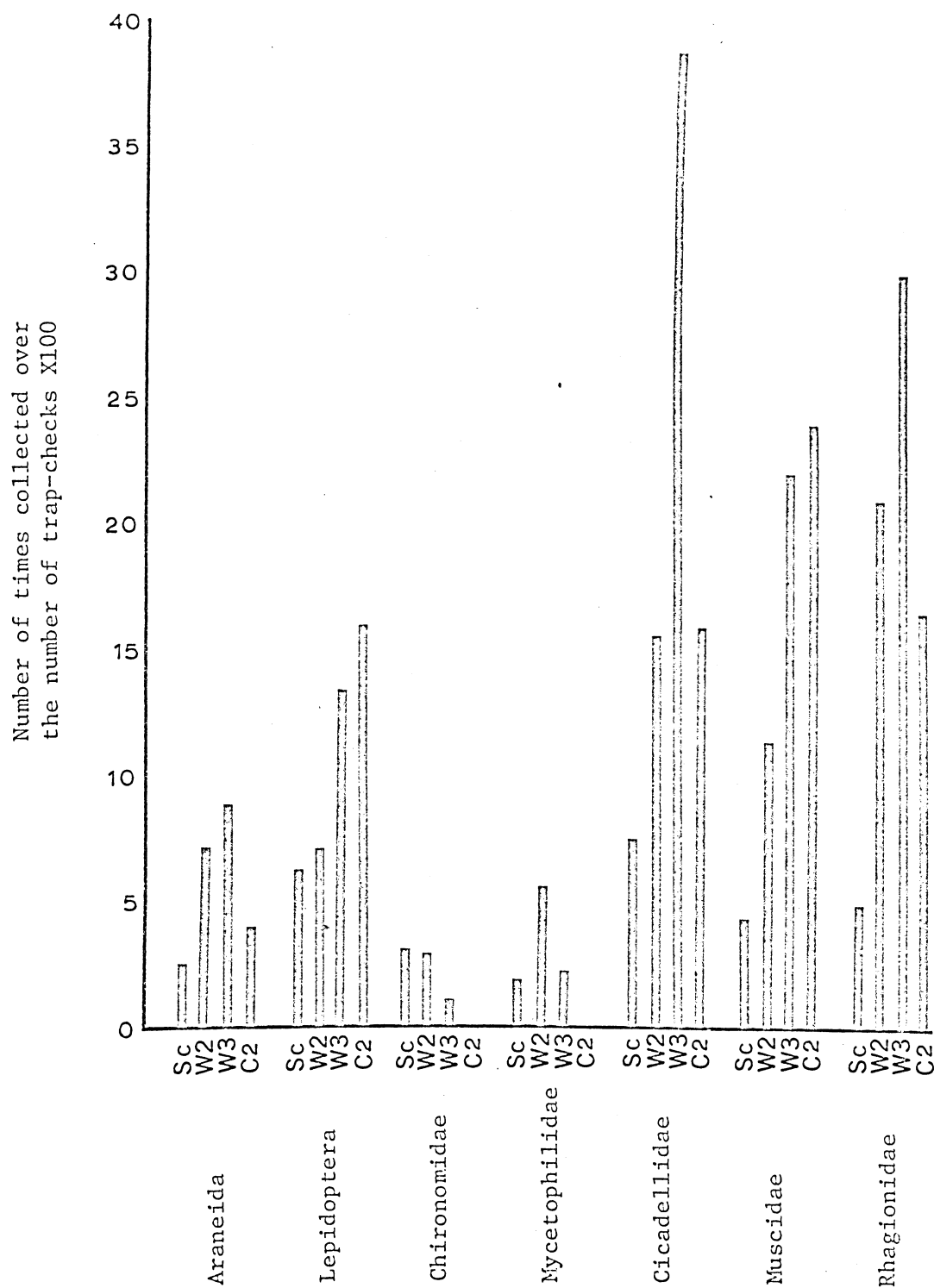Wound traps, site III; C2=Control traps, site II).

Figure 6. Per cent of individuals in the common groups
collected per trap per check in each set of
traps in 1964. (Sc=Sporocarp traps; W2=Wound
traps, site II; W3=Wound traps, site III; C2=
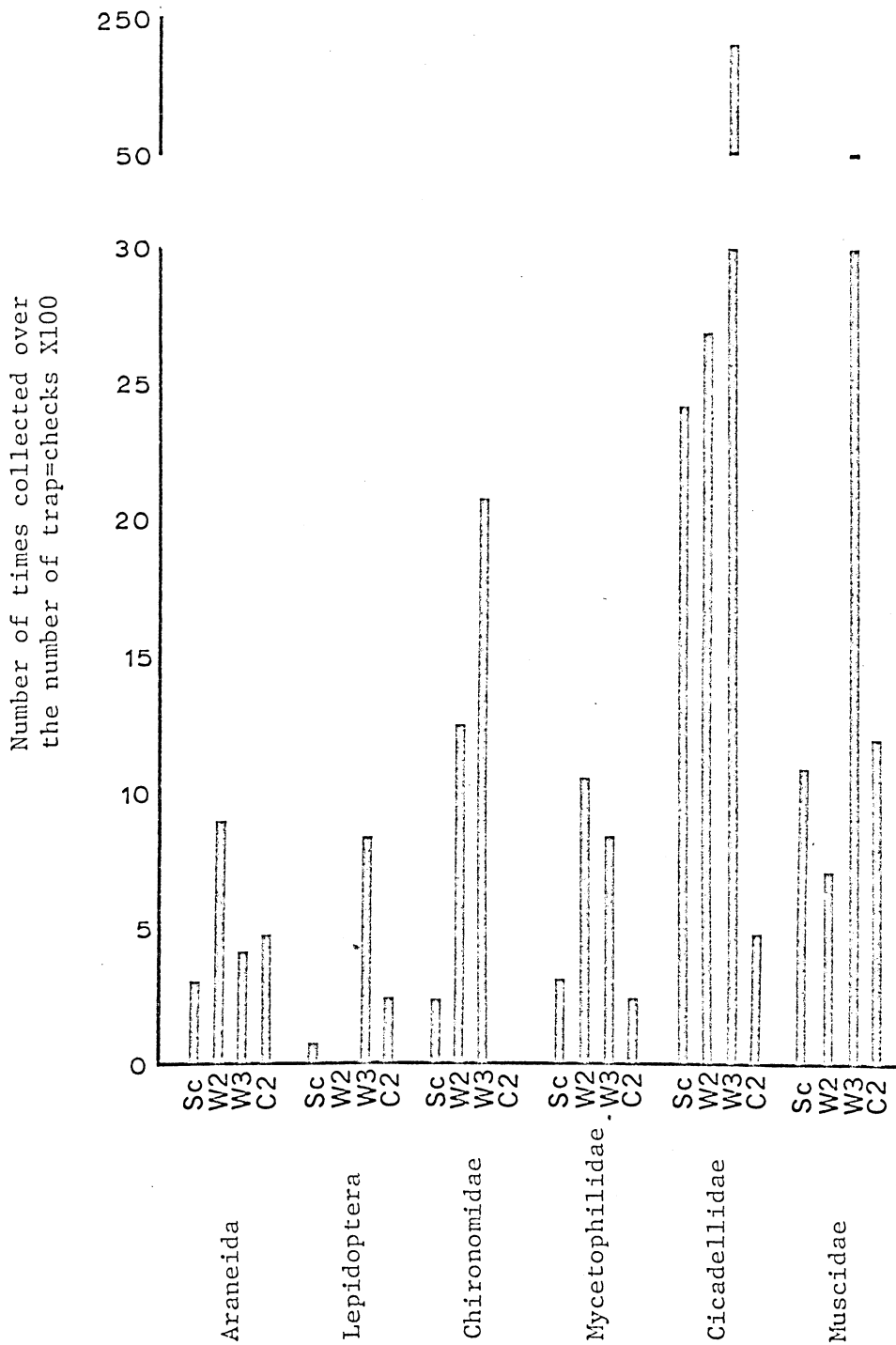Control traps, site II).

Figure 7. Per cent of individuals in the common groups
collected per trap per check in each set of traps
in 1965. (Sc=Sporocarp traps; W2=Wound traps,
site II; W3=Wound traps, site III; C2= Control
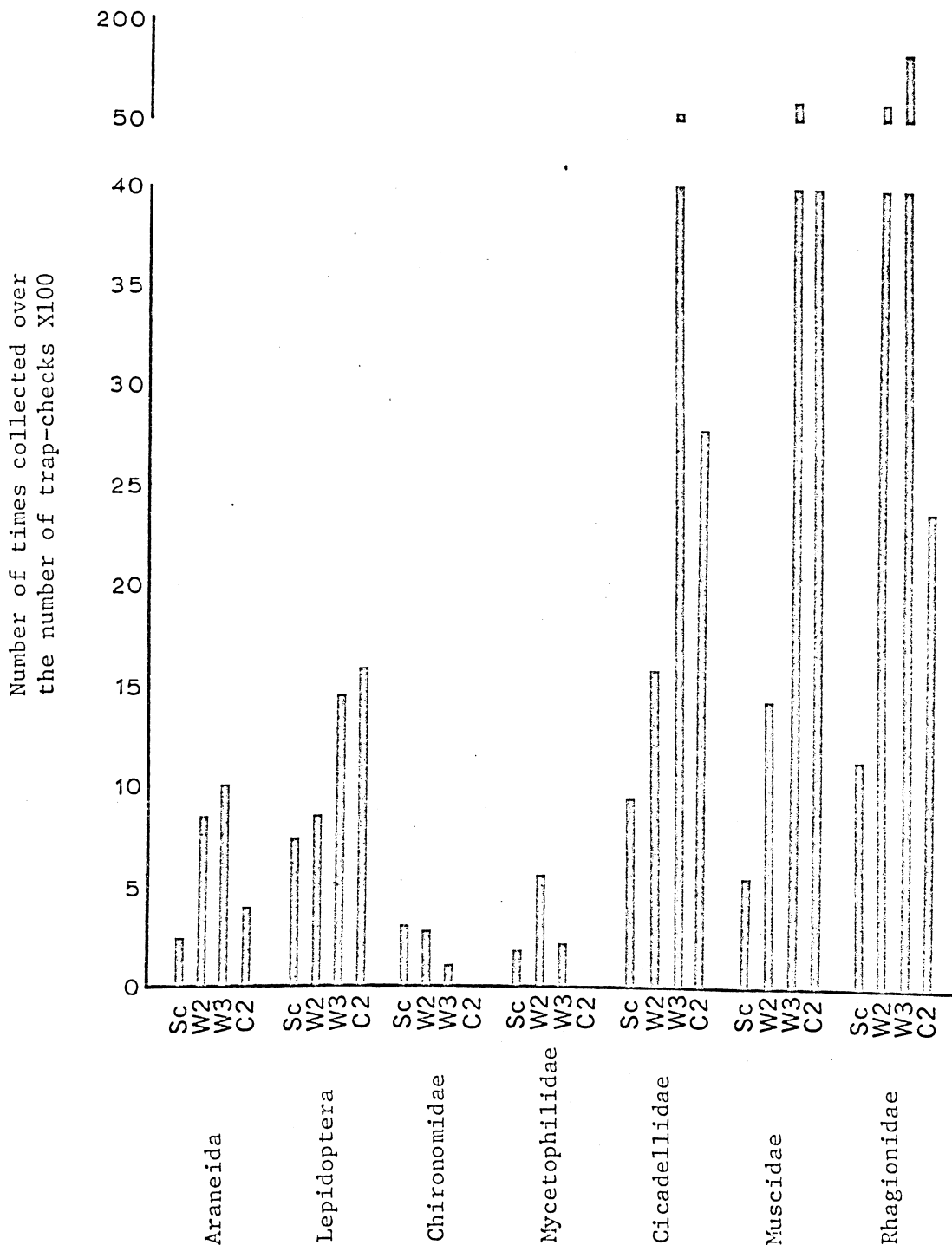traps, site II).

Figure 8. Per cent of times each common group appeared in two sets of traps in 1964. (Sc=Sporocarp traps; W2=Wound traps, site II; W3=Wound traps, site III; C2=Control traps, site II).
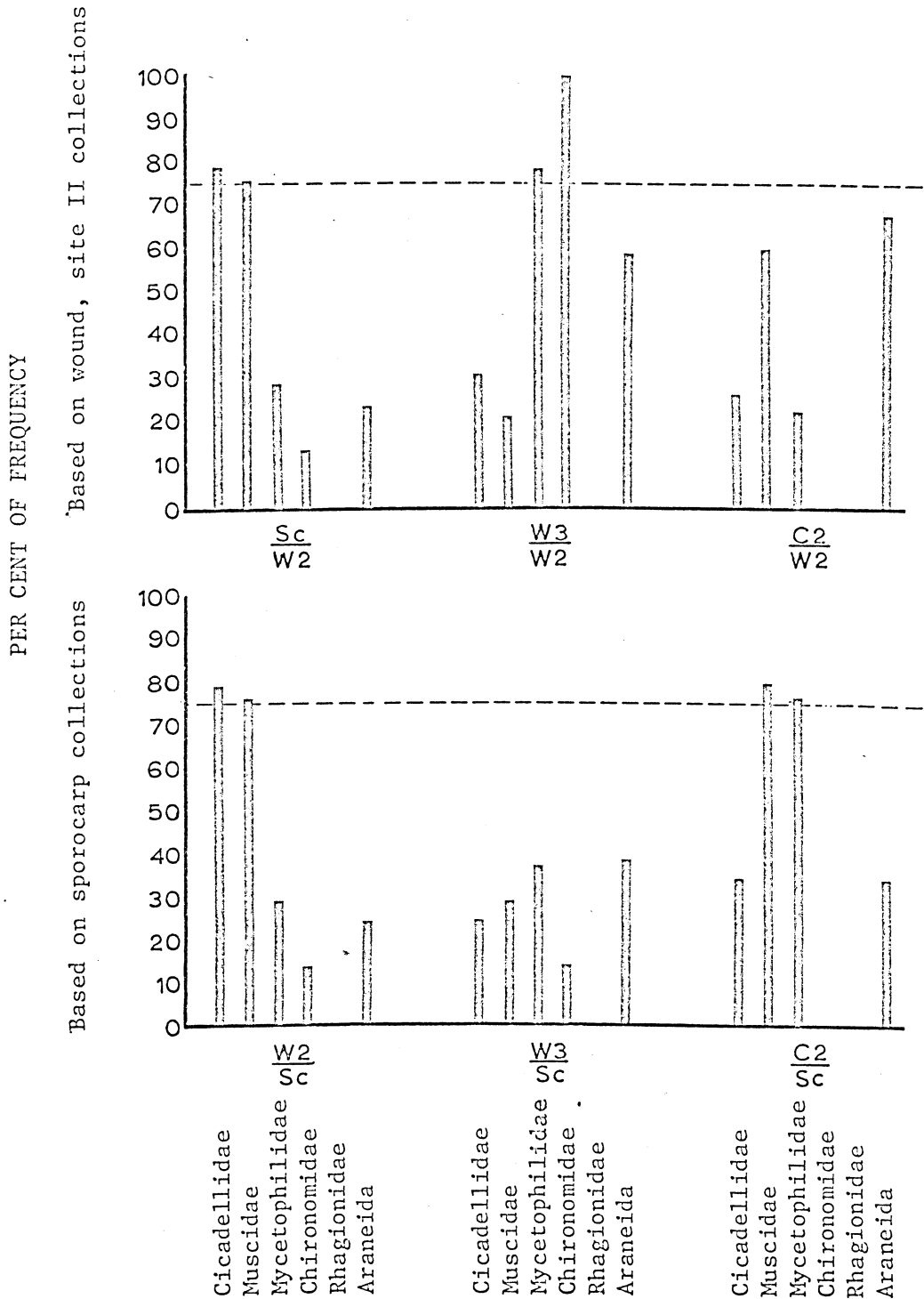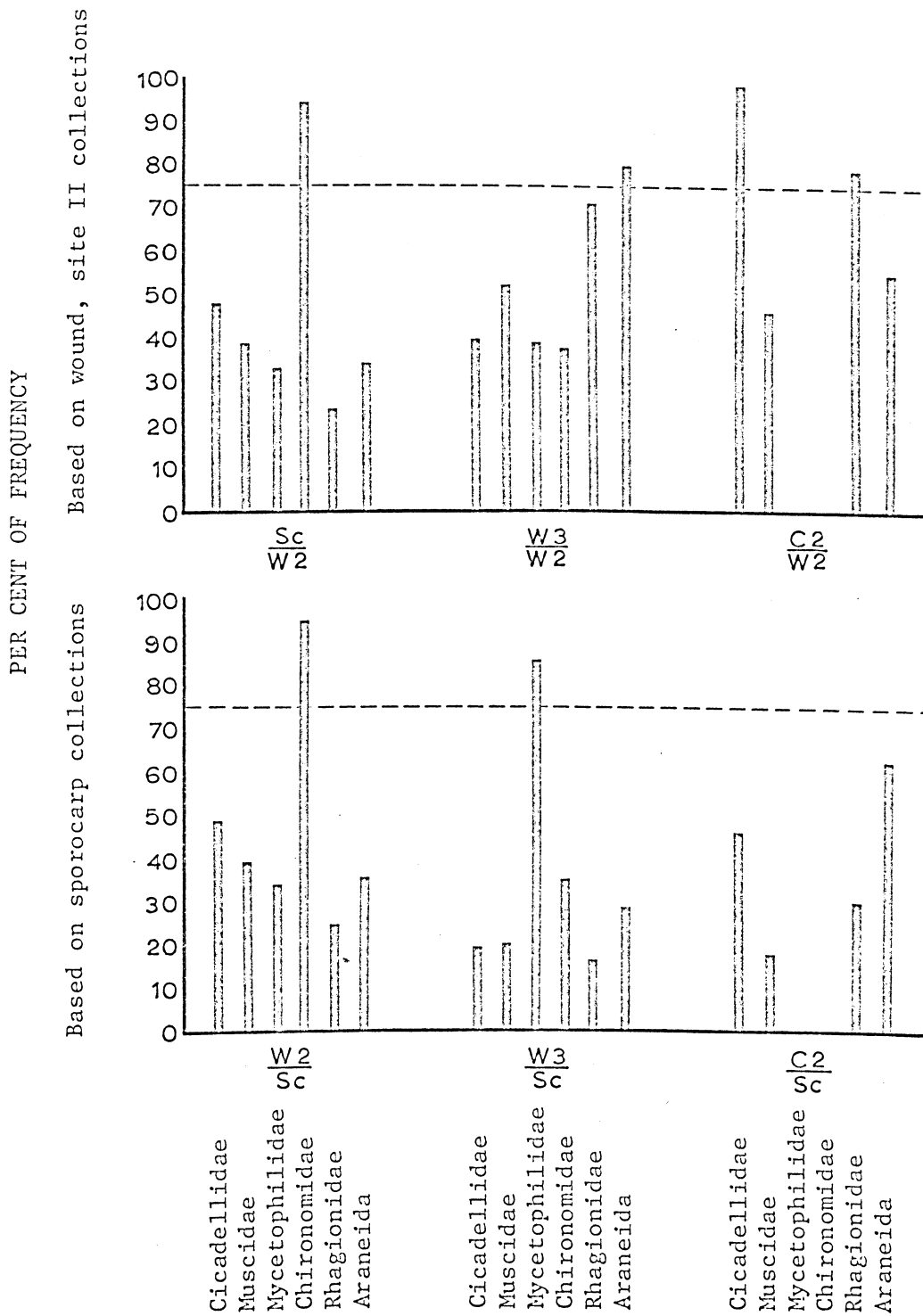
Figure 9. Per cent of times each common group appeared
in two sets of traps in 1965. (Sc=Sporocarp
traps; W2=Wound traps, site II; W3=Wound traps,
site III; C2=Control traps, site II).

PER CENT OF FREQUENCY

Based on wound, site II collections

Based on sporocarp collections

group indicates that it was attracted fairly equally to the two

sets of traps being compared. Thus, it is assumed that some

individuals of a group, appearing in two sets of traps at a 75 or

higher per cent frequency, would be likely to visit traps in both

sets during their life span.

The results, however, for the two summers were not consistent.

In the comparisons between the wound and sporocarp traps in Site II

the Muscidae and Cicadellidae were collected a high per cent of

frequency in 1964, whereas only the Chironomidae show a high per

cent of frequency in 1965. In the comparisons between the wound

traps in Site III and the sporocarp traps, all the groups showed

generally a low percentage of frequency in both summers except the

Mycetophilidae in 1965. In the comparisons with the control traps

it appears that Muscidae and possibly the Cicadellidae were being

attracted to the tree banding compound as well as to the wounds

and sporocarps. As one might expect, the groups collected in the

wound traps in Site III for both years generally had higher per

cents of frequency with the wound traps in Site II than they had

with the sporocarp traps.

The number of families each of the sets of traps collected in

common are compared in Figures 10 and 11. The lines in each sec-

tion of the graphs show the per cent of families three sets of

traps had in common with the fourth, based on the total number of

families collected in the latter. The solid lines represent the

per cent based on the total number of families collected and the

broken lines represent the same for the families collected more

Figure 10. Per cent of families collected in each set of
traps in 1965 in common with families collected
in the other three sets of traps. (Sc=Sporocarp
traps; W2=Wound traps, site II; W3=Wound traps,
site III; C2=Control traps, site II).
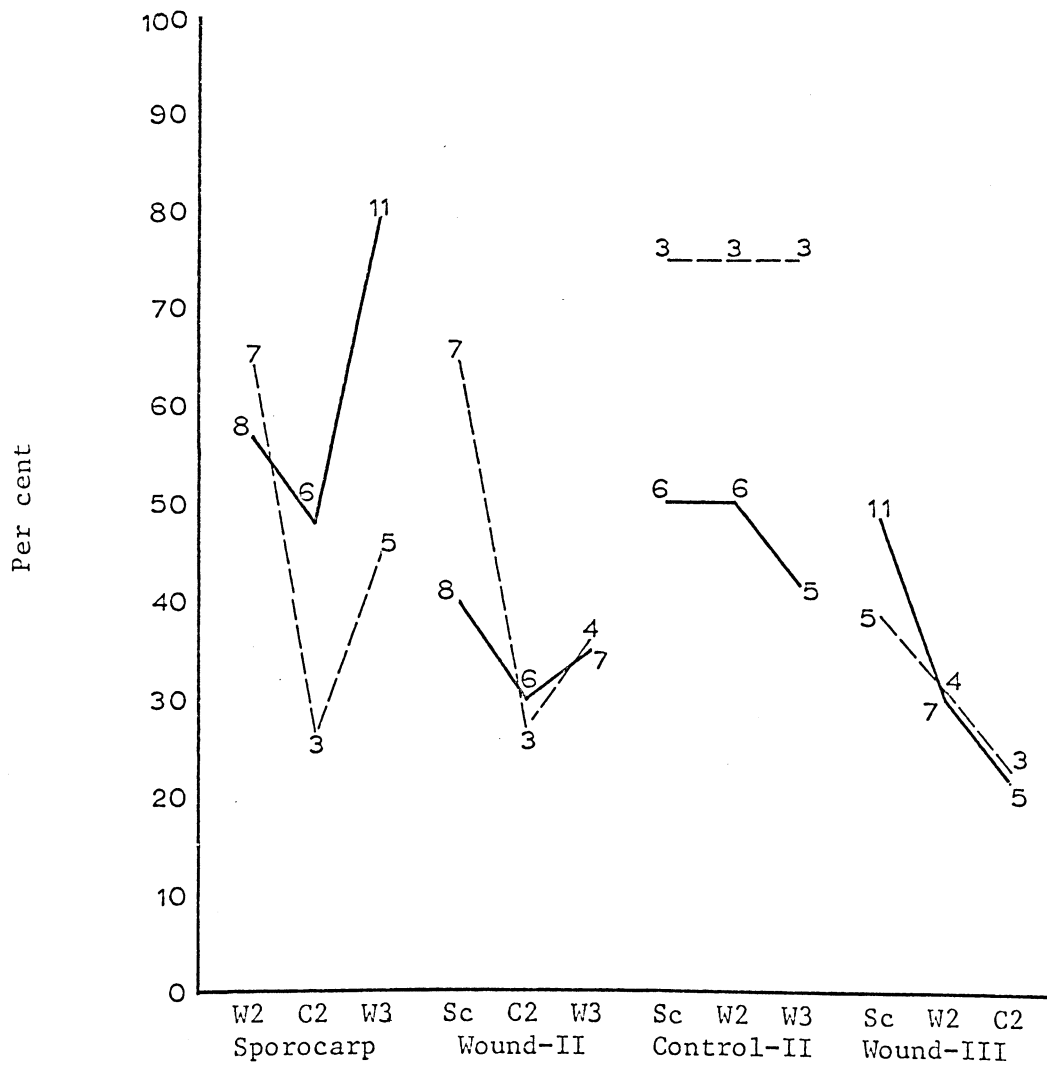
	Sporocarp	Wound-II	Control-II	Wound-III
Total families	14	20	12	23
Families coll. more than once	11	11	4	13

Figure 11. Per cent of families collected in each set of
traps in 1964 and 1965 in common with families
collected in the other three sets of traps.
(Sc=Sporocarp traps; W2=Wound traps, site II;
W3=Wound traps, site III; C2=Control traps,
site II).
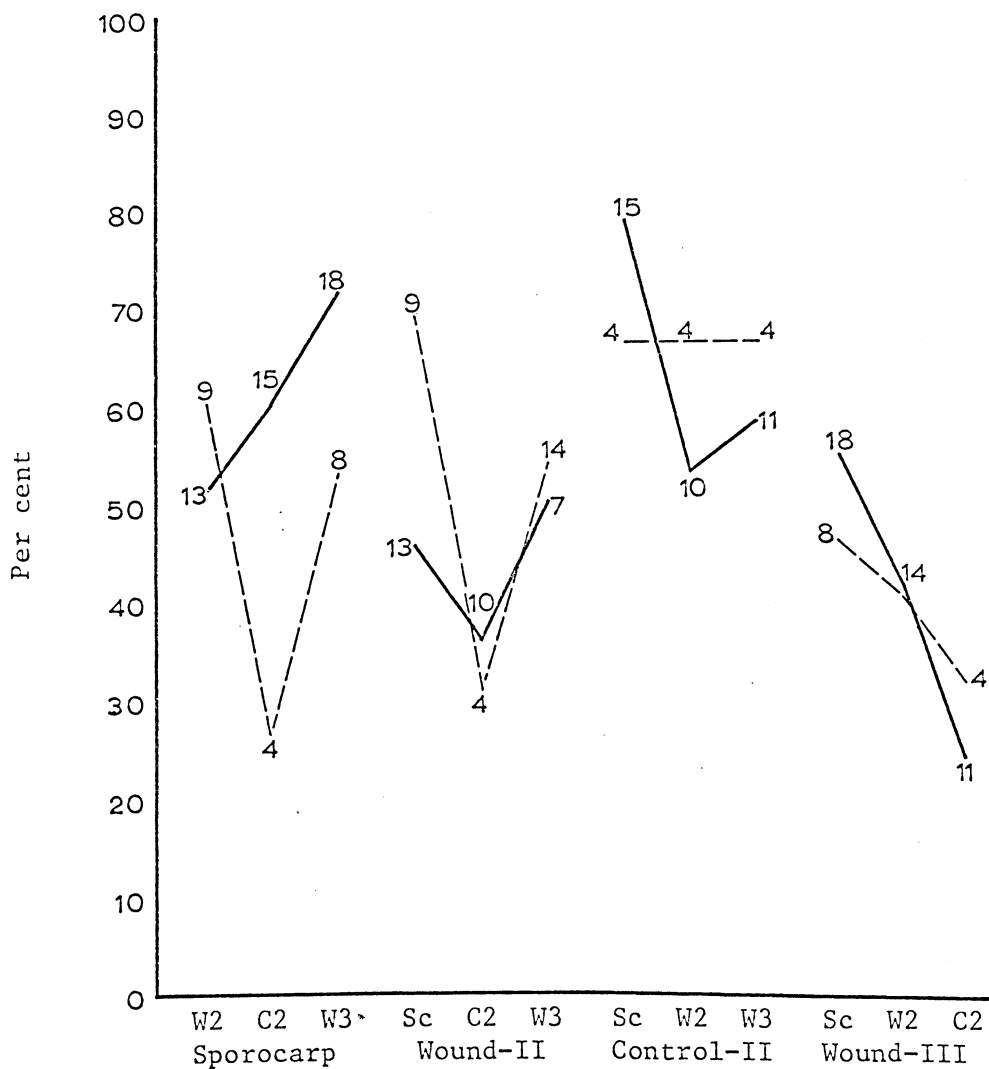
	Sporocarp	Wound-II	Control-II	Wound-III
Total families	25	28	19	33
Families coll. more than once	15	13	6	17

than once. The figures in parenthesis indicate the actual number of families involved. These results are generally consistent with those of the previous two analyses. Also the results for 1965, and 1964 and 1965 combined were quite similar. The number of families collected by both the sporocarp and wound traps was relatively high. The control traps had few families in common with the other three sets of traps. Because of the smaller number of families trapped in the controls, the other sets of traps had high values for the per cent of common families when compared on the basis of the total number of families collected in the control traps.

Discussion

Initially, the three groups, Cicadellidae, Muscidae, and Rhagionidae appear to be the most likely disseminators by the fact that they were trapped in much greater numbers than the other groups. It is assumed that the larger numbers indicate (1) that there is a greater chance a single individual will visit both the source of inoculum and a suitable inoculation site during its life span, and (2) that more inoculum would be transported. Also, the more times a particular group is trapped the less likely a high (or low) "per cent of frequency" value would be a coincidence or accident.

Of the three groups the Rhagionidae (probably mostly _Rhagio_ spp.) are possibly the least likely to be disseminators mainly because they were trapped much less frequently in the sporocarp traps than they were in the wound traps (see Figs. 5 and 7).

Also rhagionids are considered to be predators both in the larva
and adult stage which may indicate that the adults were being
attracted to the small insects caught in the tree banding com-
pound rather than to the sporocarps and wounds themselves. The
fact that they were trapped in the control traps in large numbers
is consistent with this idea. Thus, based on this information,
it seems unlikely that rhagionids are disseminators of wood deday
fungi.

It is difficult to draw conclusions concerning the Muscidae,
because of the inconsistencies in the results from the two summers.
Considering the "percent of frequency" results of the two summers
together, muscids were attracted to both the wound and the sporo-
carp traps in site II with at least a 50 per cent frequency.
Muscoid Diptera were also frequently observed in the field making
brief visits to both wounds and sporocarps. Most likely these
flies were attracted to the moist surface of the wounds as a pos-
sible source of food. Muscoid flies are attracted to many types
of soft, fleshy fungi, but it is not known whether they derive any
benefit from the hard, woody bracket fungi. Possibly they serve
as nothing more than a landing site.

These results implicate the Muscidae as possible disseminators,
and this group does seem to be particularly well suited for the
dissemination of fungi because of their mobility and "hairiness".
However, the fact remains that Muscidae were trapped in the control
traps in greater numbers than any other group, both in 1964 and in
1965. This may indicate that muscids were being attracted to the

tree banding compound itself, rather than to the wounds or sporo-carps. Therefore, it can not be concluded that muscids are dis-seminators, but they would certainly be a group to take into consideration in any further work on this problem.

The Cicadellidae also had a high "per cent of frequency" between the wounds and sporocarps in 1964, but, as with the Muscidae, this dropped off in 1965. The Cicadellidae, on the other hand, were collected much less frequently in the control traps than were the muscids. Thus, the data suggest that the Cicadellidae are the most likely disseminators of wood decay fungi of all the arthropods collected in the traps. Cicadellids were very numerous and they appeared in both the sporocarp and wound traps more than 60 per cent of the time over the two summers. How-ever, important questions remain unanswered and proof of any actual dissemination by these insects would involve much further work.

As with some of the other groups trapped in high numbers, but especially in the case of the Cicadellidae considering their nor-mal feeding habits, one can only speculate as to why they were attracted to the sporocarps and wounds in such high numbers.

Interesting comparisons can be made between my collections and those of Brues (1933). Working on a problem entirely unrelated to insect dissemination Brues trapped a total of 22,938 arthropods (over 21,000 of them insects) in tanglefoot fly paper sheets tacked to trees in forests (coniferous plus mixed coniferous and hardwoods) in northern Massachusetts. His collections were made in several locations from May through September, 1930. Despite

the differences in location and type of forest stand in which the

two collections were made there are surprising similarities in

the results. All the groups collected in high numbers in my traps

including the Chironomidae, Mycetophilidae, and Araneida were also

collected in relatively high numbers in Brues' traps (Phoridae was

the only family collected in high numbers by Brues that did not

appear in my traps). Brues assumed that the arthropods were not

attracted to the tanglefood but were caught accidentally. In

either case this puts the validity of the sporocarp and wound trap

collections into further doubt. Certainly if this work was to be

repeated other means of trapping the insects should be tried, or

at least a large number of control traps should be established.

The possibility of dissemination by the groups collected in

smaller numbers can not be ruled out even though no definite con-

clusions can be made from these data. The Mycetophilidae, some of

which are known to be associated with fungi might be a group to

investigate further. Interestingly, the mycetophilids were found,

not only in site II where one would expect to find them, but also

in site III where no sporocarps of wood decay fungi were known to

exist.

The comparisons between the insects trapped in the sporocarp

and wound traps in site II have been given the most significance

in these analyses. In a practical sense, dissemination within

this particular stand might not have any value, because most of

the trees already had heart rot. It is assumed that this does not

affect the results and that insects would appear at both wounds

and at sporocarps in much the same way in stands with a lower
level of heart rot. Dissemination from site II to site III would
have practical value in this case, but because the two stands are
350 yards apart, there is a reduced chance that it does occur.
Some groups, especially the Cicadellidae, were extremely common
in the wound traps in site III (see Figs. 4 and 5), and dissemi-
nation into this area might have occurred were the two stands
closer together.

Several aspects of this work have limited the conclusions
which can be drawn from the data. Determining the arthropods
caught in the traps simply to family, or in some cases to order,
was a severe limitation. However, the large number of arthropods
involved and the fact that many of the specimens were in poor con-
dition after being removed from the tree banding compound, would
have made further determination a difficult and time consuming
task. Insects landing on the wound surface itself and the pore
surface of the sporocarps were not caught because these areas
could not be covered with the tree banding compound. It is not
known whether large numbers of insects were missed because of this.
Also having more than one sporocarp trap per tree and only one
wound trap per tree may have biassed the results, and comparisons
might have been more meaningful if more control traps had been
established. Comparing the results would have been simpler if
an equal number of traps had been set up in each of the four sets
of traps.

Isolations of Wood Rotting Fungi From Insects

A part of the sporocarp and wound trap experiment was the isolation of any wood rotting fungi from a sample of the insects caught in these traps. The main purpose of this was to determine whether the insects were carrying inoculum to the wound traps.

Methods

The basic procedure was the insertion of the insects into holes in aspen blocks which were then stored in a saturated environment. The aspen wood was to act as a selective medium for any wood rotting fungus borne by the insect.

The aspen blocks used had 1 inch dimensions and a $\frac{1}{4}$ inch hole drilled 1/2 to 2/3 of an inch into one side. In 1964, seasoned or dry blocks were used, but these were soaked in water from 1 to 3 weeks before insertion of the insect, to provide moist wood for the fungi. In 1965, freshly cut green aspen obtained from a local lumber yard was used. The blocks were stored in plastic bags and were kept frozen until needed. Arthropods representing many of the groups collected from the various wound and sporocarp traps were inserted into the block, and the hole was sealed with a size 00 cork. From one to several arthropods of the same group were put in each block. Inoculated blocks were then sealed in $\frac{1}{2}$ pint jars with a small amount of water added to maintain a saturated environment. These were stored for several months to give any wood decay fungi an opportunity to grow. Neither the jar, the block, or the cork was autoclaved. Forceps used to handle the

insects were flamed between each use. A total of 152 blocks were
inoculated over the two summers.

After a 3 to 6 months development period, the blocks were
checked for external indications of a wood rotting fungus. The
best indication was the presence of a lush growth of white myce-
lium on the aspen block. Blocks which had small amounts of white
mycelium growing on them especially around the opening of the hole
and cork, whitish markings and circular light patterns at the end
opposite the hole, and discolorations such as dark lines and mark-
ings were also selected. Only these blocks which showed these
visible signs were further checked by isolating the fungus on the
block for identification.

The following procedure was used in isolating the fungi from
the suspected wood blocks: If the block had a good growth of white
mycelium growing on the outside, small parts of this were plated
out directly. The block was then split open through the axis of
the hole, usually slightly off center, with a hand ax. Pieces of
the freshly exposed wood close to the hole were removed with a
razor blade. This wood was often darkened or discolored somewhat.
Three pieces of the wood, each treated differently, were plated
out. One piece was plated directly, another was flamed briefly in
a bunsen burner and then plated, and the third was dipped in 95%
alcohol, flamed, and then plated. All the tools used in handling
the blocks, the mycelium, and the pieces of wood were flamed be-
tween each use. The isolations were made on 2 per cent malt agar
and subsequent isolations were made until the suspected fungus

was growing in pure culture.

Results and Discussion

From the 1964 block inoculations, nine (out of 46) blocks had external characteristics which indicated the possible presence of a wood decay fungus, including a few with lush growths of white mycelium. Only seven (out of 106) inoculations in 1965 had favorable external characteristics. Fungi isolated from blocks inoculated in 1964 have not been identified exactly, but a wood rotting fungus was obtained from a few of them. No wood rotting fungi were isolated from the blocks inoculated in 1965. _Trichoderma_ sp. was the predominant fungus obtained from the blocks from which isolations were made in both 1964 and 1965.

Some preliminary work in May, 1964, using the block technique and a variety of insects from rotting logs produced better results. Of 20 inoculated blocks, seven had good external indications of a wood decay fungus. A wood rotting fungus (exact identification not made) was isolated from one block inoculated with an ant.

It would seem that more of the insects collected in the sporocarp and wound traps would be carrying spores of wood decay fungi than was indicated by the block technique. Some blocks inoculated with _B. cornutus_ which were thoroughly covered with spores of _F. applanatus_ showed no signs of the growth of this fungus after many months. Most of the blocks sealed in $\frac{1}{2}$ pint jars acquired a disagreeable odor indicative of bacterial or yeast growth. This may have killed or suppressed any wood rotting fungus that may have been present. Possibly the jars were kept too moist. It is also

interesting that the few wood decay fungi that were isolated were from the seasoned aspen blocks and not from the fresh ones used in 1965. It seems possible that this technique could be perfected so that consistent and reliable results could be obtained.

Dissemination Experiments

The third part of the investigation of random dissemination was a test of the ability of the insects and arthropods commonly caught in the wound and sporocarp traps to carry fungus spores. Also, muscid flies were used to test the inoculation of wounds by only a brief period of insect-to-wound contact, and Bolitotherus cornutus was used to test the ability of a hard, rough-surfaced insect to retain spores on its body for relatively long periods of time. The Deuteromycete fungus, Hormodendrum resinae, was used in this experiment, rather than a wood rotting fungus, because of its ability to grow on creosote agar to the exclusion of almost all other organisms. This property of H. resinae makes it well suited for this work since it can be reisolated with relatively rapid results and without concern about contaminates. The fungus is not naturally found in the air or in the soil, but is found growing on asphalt, resinous bark, and on wood impregnated with coal tar or creosote (Christensen et al., 1942).

The purpose of the experiment with H. resinae was the testing of various arthropods as disseminators and specifically to determine whether: (1) arthropods readily pick up fungus spores on their bodies, (2) the spores are retained for extended periods

of time, and (3) the spores are deposited when the arthropods come in contact with a suitable inoculation site. The spores of H. resinae are dry and dust-like, as are those of Fomes spp., so it is assumed that the results from the former would apply also to these wood decay fungi.

Methods

Creosote agar (0.5 to 0.75 per cent creosote) and stock cultures of H. resinae were obtained from the Plant Pathology Department, University of Minnesota. Live insects representing the groups commonly collected in the wound and sporocarp traps were contaminated by putting them in 300 ml. erlenmyer flasks containing cultures of H. resinae. The insects were left in the flasks for approximately 2 minutes, or until they had made contact with the fungus spores. They were then put into plastic shell vials (1 inch by 3 5/8 inches). The vials had previously been glued to a 2 inch strip of heavy cellulose acetate through which a 1 inch circular hole had been cut to match the 1 inch hole of the shell vial. This provided a means of tacking the vial to the wounds made on aspen trees. The bottom of the vials were perforated with 30 to 35 pin holes to allow some ventilation. This is similar to the technique used by Dorsey et al. (1953) in their study of oak wilt transmission by nitidulid beetles.

Several mature aspen in site I which bore no sporocarps of wood decay fungi were selected, and notches were cut into the trees with a small tree saw just prior to the attachment of a vial containing a contaminated arthropod. The vials were held in place

with two carpet tacks in a position such that the open end of the
vial was against the exposed surface of the tree's sapwood, and
was lower than the closed end, assuring contact between the insect
and wood (Fig. 12). Arthropods used were contaminated the same
day they were attached to the wounds. The vials containing the
arthropods were left in place from 2 to 13 days before isolations
were made from the wound surface.

To isolate any H. resinae inoculum from the wound, the vial
was removed and a few small shavings were taken from the wood sur-
face where it had been in contact with the arthropods. Shavings
were placed directly on creosote agar in petri dishes. The razor
blade and the forceps used to remove and handle the shavings were
flamed between each use. The plates were examined periodically
and the amount of growth of H. resinae was recorded. A control
vial containing no arthropod was attached to a wound in the same
manner as the others.

Muscid flies were used for the experiment involving brief
periods of contact with the wound, because they are commonly seen
in the field landing on both sporocarps and wounds for brief
periods of time. Flies were collected, contaminated, and allowed
to remain on the wounds for 30 seconds, 5 minutes, 10 minutes, and
for longer periods.

The beetle, Bolitotherus cornutus, was used in the experiment
involving spore retention by the insect, because they are long
lived and hardy. Six beetles were all thorough contaminated on
August 16, 1965, and stored in a common container. Subsequently,

after increasingly longer intervals of time in the container (30

minutes: moved to a small

vial and   wound on an aspen.

Results

H.   all the wounds

in contact   ated from the con-

trol woun   ested (listed in

table 6)   spores. The experi-

ment with   capable of inocu-

lating th   ) contact period

used. Th   in contact with

the flies   and 3 weeks, but

was not i   periment with g.

cornutus   wounds declined

fairly rapidly with time spent in the container after they had

been contaminated (see table 5). Isolations from wounds which

had been in contact with beetles stored 1 and 2 weeks after con-

Figure 12. Plastic shell vials, showing method of attach-
ment to the tree.

agar. However, numerous colonies of the fungus were obtained when

the beetles themselves were plated (after being killed) after they

had been contaminated and subsequently stored for 4 and 5 weeks.

This showed that the beetles may be carrying fungus spores but

still not deposit any, or enough, inoculum on the wood surface to

be detected by reisolation. This was probably caused by the

beetles rubbing against each other, the container, and the pieces

of _T. applanatus_ context (included for food) resulting in a loss

after increasingly longer intervals of time in the container (30 minutes; 1 day; 1, and 2 weeks), a beetle was removed to a shell vial and attached as described above to a fresh wound on an aspen.

Results

H. resinae was successfully reisolated from all the wounds in contact with the arthropods, but was not isolated from the control wound. Thus, all eleven arthropod groups tested (listed in table 4) retained and deposited the H. resinae spores. The experiment with the muscid flies showed that they are capable of inoculating the wound during the shortest (30 seconds) contact period used. The fungus was also isolated from wounds in contact with the flies for 5 min., 10 min., 2 days, and 1, 2, and 3 weeks, but was not isolated from the control wound. The experiment with B. cornutus showed that their ability to inoculate wounds declined fairly rapidly with time spent in the container after they had been contaminated (see table 5). Isolations from wounds which had been in contact with beetles stored 1 and 2 weeks after contamination showed only traces of fungus growth on the creosote agar. However, numerous colonies of the fungus were obtained when the beetles themselves were plated (after being killed) after they had been contaminated and subsequently stored for 4 and 5 weeks. This showed that the beetles may be carrying fungus spores but still not deposit any, or enough, inoculum on the wood surface to be detected by reisolation. This was probably caused by the beetles rubbing against each other, the container, and the pieces of F. applanatus context (included for food) resulting in a loss

Table 4. Arthropod groups contaminated with H. resinae and
          placed in contact with fresh wounds on aspen.

Family or Order	No. of insects in vial	Date vial attached to wound	Date of isolation from wound
Muscidae	2	July 6	July 19
Lampiridae	2	July 6	July 19
Elateridae	2	July 6	July 19
Rhagionidae	2	July 7	July 19
Muscidae	1	July 29	July 31
Tipulidae	1	July 29	July 31
Cicadellidae	1	July 29	Aug. 2
Tipulidae	1	July 29	Aug. 2
Phalangida	1	July 29	Aug. 2
Araneida	1	Aug. 15	Aug. 17
Miridae	1	Aug. 15	Aug. 17
Formicidae	1	Aug. 15	Aug. 20
Control	-	July 31	Aug. 23

Table 5. Isolations from wounds (and beetles) exposed to B. cornutus after increasing lengths of time after contamination. All beetles contaminated with H. resinae on August 16, 1967.

No. of wound or beetle	Time between contamination and contact with wound*	H. resinae reisolated (+) not reisolated (−)
1, wound	30 min.	+
2, wound	1 day	+
2, beetle	1 day	+
3, wound	1 week	+ (trace)
3, beetle	1 week	−
4, wound	2 weeks	+ (trace)
4, beetle	2 weeks	+
5, beetle	4 weeks	+
6, beetle	5 weeks	+

*Or creosote agar in the case of the beetles

of the excess spores on their bodies in the week to 2 week period. However, because their integument is very rough and irregular, enough spores must have been left in protected places so that even after 5 weeks in the container the fungus developed when the beetles themselves were plated.

Discussion

These results clearly show that the arthropods tested can retain spores of H. resinae on their bodies (for at least 2 weeks in the case of B. cornutus) and can deposit these when in contact with a wound. It seems probable that spores of wood rotting fungi would be retained and deposited in much the same manner. Despite the questions surrounding the sporocarp and wound trap experiment, it seems likely that some insects would randomly visit both the sporocarps and the wounds. And, if the insects can become contaminated in some other way, such as from spores in the air, the sporocarp visit would not be necessary.

Assuming that arthropods are contaminated with inoculum of wood rotting fungi, an important question still unanswered is whether a wood rotting fungus would become successfully established after the deposition of the inoculum. Many factors, such as the amount of inoculum, or the physical and physiological condition of the wood, are important to the successful establishment of a wood rotting fungus. The failure of the "block technique" leaves this unanswered. It should be restated at this point, however, that even had all three indirect experiments on random dissemination been "successful", one could still only conclude that

dissemination seemed very likely.

Since the experiments using H. resinae could not show that insects successfully inoculate wounds with wood decay fungi, similar experiments were set up using B. cornutus contaminated with the spores of F. applanatus. It should be stated at the outset that the final results of this are not known at the time of writing. However, the procedures used will be briefly described.

Methods

Live B. cornutus beetles were collected from active F. applanatus sporocarps. Beetles were selected which were obviously well covered with the spores of this fungus as indicated by their rusty-brown color. They were placed in plastic shell vials (one beetle per vial) identical to those used in the previous experiment, and were attached to aspen trees and logs in the following four situations:

(1) In site III a vial was attached to a notch cut 1 foot from the ground in each of 13 aspen (d.b.h. between 4 and 6 inches). The beetles were collected July 27, 1965, and four were attached the same day (plus one control vial) to freshly cut notches. Subsequently, two more beetles were attached at weekly intervals to freshly cut notches in each case.

(2) On August 11, 1965, three beetles, collected the previous day, were attached to notches on three live, mature aspen trees in site II. The notches were all about 1 foot from the ground, and the trees bore no visible sporocarps.

(3) The third group of three vials was attached to an aspen log introduced into site I which had been cut from a live tree approximately one year before.

(4) The final group of three vials was attached to a dead aspen about 35 feet high. The stump had been a live tree earlier in the year, but the top had broken off during a wind storm sometime between May 21 and June 12, 1965. The vials were attached about 2 feet from the ground and a control vial without a beetle also was attached.

Results and Discussion

On October 30, 1965, samples were taken from the wood in the notches of the young aspen in site III to which vials had been attached on July 27, August 3, and August 10, 1965. Isolations were made from these in the laboratory, and no wood rotting fungi (no Basidiomycetes) were obtained. Assuming that viable spores of F. applanatus were transferred to the wounds in sufficient numbers, several factors could explain its apparent absence. (1) The fungus had not had enough time to become established in the wood. (2) The fungus was suppressed by the young, healthy aspen. (3) The isolation technique was inadequate. Probably 3 months was not long enough for a wood rotter to become established but all three factors could have been involved.

SECTION II. STUDIES ON <u>BOLITOTHERUS</u> <u>CORNUTUS</u> AS A

POSSIBLE DIRECT DISSEMINATOR OF WOOD ROTTING FUNGI

It is well known that the forked fungus beetle, <u>Bolitotherus</u>

<u>cornutus</u> (Panzer) (Tenebrionidae), is associated with many poly-

pores in the wooded areas of the eastern two thirds of the United

States. The investigation of the behavior and movements of this

beetle was undertaken mainly to determine whether it might be a

direct disseminator of the fungus on which it lived.

In the Itasca area the beetles are most commonly associated

with the sporocarps of <u>Fomes</u> <u>applanatus</u>. Beetles are often so

covered with the rusty-brown spores of this fungus that they appear

this color rather than their natural color. Thus, there is little

doubt that they meet the first requirement of a good disseminator,

i.e., a large amount of inoculum adheres to their bodies because

of their activities. Therefore, their biology and movements were

observed for two summers to determine whether they carry the inocu-

lum to suitable inoculation sites.

The investigation of <u>B</u>. <u>cornutus</u> is divided into four main

sections as follows: (1) a description of its life cycle, taken

mainly from the literature; (2) a discussion of its behavior based

on the literature and my own observations, (3) an analysis of its

movements based on marked individuals, and (4) a description of

dispersal experiments. The literature pertinent to these aspects

will be discussed in each section.

Description of the Study Area and Methods

In a maple-basswood stand near the biology station (Site I) several large aspen logs bearing active sporocarps of Fomes applanatus were concentrated in a relatively small area. The fungi in turn supported large populations of B. cornutus.

The specific study area was a low, open area bordered by two large up-rooted aspen trees running nearly parallel with each other in a northeasterly to southwesterly direction. (Northeast and southwest logs hereafter referred to as north and south logs, respectively) The two logs were 44 feet apart at their butt ends and approximately 35 feet apart where their first branches appeared. Their diameters, measured 10 feet from the estimated original ground line, were 20 and 18 inches for the north and south logs respectively.

When first discovered in July, 1964 the two logs bore a total of 37 active F. applanatus sporocarps (17 and 20 on the north and south logs, respectively) ranging in size from 2 inches by 3 inches to $7\frac{1}{2}$ inches by 12 inches. (The first dimension is the maximum extension outward from the log and the second is the maximum width at the base.) In addition to the primary sporocarps, the logs also bore about 85 very small to medium sized sporocarps in various stages of deterioration. Nine of the 85 represented small, newly emerging sporocarps, either at a new site or at the site of an older one which had been broken off. Most of the other 76 were dead or nearly dead and had been partially or completely destroyed by the beetles. These were no longer sutiable breeding

sites for B. cornutus.

The trees had probably been down for about 6 to 8 years in 1964 judging from the age of the larger sporocarps borne by both logs. The bark was still intact and fairly tight against the wood on both logs. The north and south logs were separated by the southwest end of a low area which was characterized by a grass and herbaceous plant cover and the absence of any large trees. The depression was free from standing water when first located in the middle of July, 1964. It was completely flooded, however, in the spring of 1965 and remained so until late June.

Two other aspen logs lay just outside the low area about 20 feet southwest of the butt end of the south log. These two logs bore only five to six active F. applanatus sporocarps. They had been down approximately the same length of time, or possibly one or two years less, as had the north and south logs. Also in Site I, 120 feet south of the aspen logs just described, was an aspen stump 18 feet high and with a d.b.h. of 18 inches. This stump bore about 12 deteriorating F. applanatus sporocarps. The population of B. cornutus associated with this stump was used in ultra-violet light and dispersal studies.

The large population of B. cornutus on the sporocarps of the north and south log were used for observations on the beetle's behavior and for following the movements of individual beetles. The primary sporocarps on each of these two logs were numbered consecutively and used as location reference points. Starting in July, 1964 the beetles on the two logs were individually marked

with a red enamel paint. The location of the beetles was recorded

every two days and observations were made of their behavior at

the same time. Because the beetles are mainly nocturnal, night

location checks and observations were started in August, 1964.

Detailed observations at night, however, were almost impossible

because the beetles proved to be very sensitive to visible light.

In 1965, to determine whether observations could be made at night,

the beetles were marked with fluorescent paint and observed with

ultraviolet light. Also in 1965, a light-proof observation hut

was constructed over a portion of the south log so that the beetles

could be observed during the day in a darkened environment. The

main purpose of the hut was to see if the beetle's activities

were governed by light.

Life Cycle

The stages in the life cycle of B. cornutus have been

described by various people as follows. The whitish eggs are

cylindrical with broadly rounded ends, and they measure 1.7 to

2 millimeters long and 0.8 to 1.0 millimeters wide (Weiss and

West, 1920b). The larvae are cylindrical with distinct, subequal

segments; the color is dirty white except for the prognathous

head, the mandibles and urogomphi. The thoracid legs are well

developed and the spiracles are anular except for a large oval

mesothoracic spiracle (Peterson, 1960).

Triplehorn (1952) (Fide, Liles, 1956) has described the

adults.

Elongate-rectangular, robuts; black to reddish-brown,
dull, lusterless; head roughly sculptured, sides elevated;
males with bifid horn on clypeus; female with two widely
spaced small tubercles on clypeus; antennae 10-segmented,
second segment small, third longer than three following
segments combined, terminal segment globular; eyes deeply
emarginate; pronotum roughly sculptured, twice as broad
as long, lateral margins broadly flattened, serrate with
variable number of rounded teeth; males with two slightly
curved horns, broader at tip, and clothed with yellow
hairs beneath, projecting forward from disc of pronotum
to well beyond head; females with blunt tubercles instead
of horns, elytra roughly sculptured, each with four rows
of large irregular tubercles and smaller ones on inter-
vales; abruptly deflected apically; epipleura entire.
Ventral surface and legs dull black, lusterless, rugose;
males with patch of yellow hairs on inner face of femora;
prosternum blunt, horizontal, its apex prominent, length
10-12 mm., width 3.5-4 mm.

Liles (1956) has worked out the life history of this beetle by
rearing them in the laboratory and by observing them in the field,
the latter in northern Michigan. The following discussion is
taken from Liles (1956) unless otherwise noted.

He states that there appears to be two egg laying seasons in
northern Michigan; one from June 15 to about July 1, and the other
from the end of July to the middle of September. B. cornutus over-
winters in both the adult and larval stage. The spring laid eggs
become adults that same summer and overwinter as adults inside the
sporocarps, in the leaf litter, under the bark, or in other pro-
tected sites close to the host fungus. Upon emerging these adults
will lay the next spring brood of eggs.

The eggs are laid singly usually on the upper surface of a
sporocarp along the concentric grooves and folds and along the
outer margin. Infrequently they are laid on the under surface on
the edge of a dead hymenial layer. Eggs appear to be never laid

on the living hymenium. (Wiess and West, 1920 and Liles, 1956).

The females cover the eggs with a brownish excrement which drys

leaving "blisters or egg capsules" clearly visible on the fungus.

These usually measure about 3.5 mm. long and 2.5 mm. wide. Liles

reported that oviposition always occurred in the laboratory be-

tween 5:00 and 7:30 p.m., and that females lay 8 to 12 eggs apiece.

Liles' description of oviposition was very much as I observed

it on August 16, 1964 and again on July 19, 1965 with the excep-

tion that on the first occasion the three females I observed were

ovipositing in complete darkness between 10:25 and 11:00 p.m.,

and on the second occasion I observed a single female ovipositing

at 3:30 p.m. The following description is based mainly on my

observation on August 16.

Two marked female beetles (#2 and #14) and an unmarked indi-

vidual had just laid single eggs with the long axis of the eggs

following the concentric grooves on the upper surface of one

sporocarp. Number 14 had completed the covering process but

remained standing motionless for some time with her abdomen near

the egg capsule. The other two females, began to move the tips

of their abdomens over the freshly laid eggs in a slight up and

down and side to side motion while, at the same time, covering

the eggs with a moist, lumpy excrement. This process continued

for about 25 minutes as each beetle built up a lumpy covering

over its egg. The dark brown material lightens in color as it

drys and hardens, forming a capsule over the egg which more or

less matches the color of the substrate. One of the females

moved away soon after completing the capsule while the other one was still in place when the observation was terminated.

By rearing the beetles in the laboratory, Liles found that the eggs hatch in 11 to 26 days (average, 16 days) and that the first instar larvae lived for about 5 days in the capsule before burrowing into the sporocarp or breaking out of the capsule. Larvae were reared through four instars with the first, second, third and fourth instar periods lasting an average of 9, 8, 11.3, 44.5, and 7 days respectively. Liles observed a fifth instar in the field.

The following information is also taken from Liles (1956). The first instar larva leaves the egg capsule and bores into the fungus either directly from the capsule or at some other point on the surface of the sporocarp and begins making small tunnels close to the surface. As the larvae grow the tunnels increase in size accordingly and eventually the context and often the old hymenial layers are extensively mined by the larvae. Dark granular frass accumulates in the tunnels as they are abandoned. Several larvae may occupy the same sporocarp and their tunnels may cross but generally each larva remains in its own tunnel system. The larva pupates in a chamber large enough for the pupa to move freely about as the latter is rather active. The pupal stage lasts an average of 11.6 days in the summer. The newly emerged adults appear somewhat reddish in color but darken to their normal color in a few days. The darkening period is often spent in the pupal chamber. The adults then emerge through the surface of the fungus

randomly, leaving holes 6 to 9 mm. in diameter. Unless the fungus
has been attacked repeatedly or is in a late stage of deteriora-
tion, the larval feeding is not visible from the outside.

Adult Beetle Behavior

General and brief comments about the behavior of Bolitotherus
cornutus appear in the writings of some of America's early ento-
mologists as well as in those of some more recent workers (Say,
1828, LeConte, 1861, Harrington, 1880, Blatchly, 1910, Edwards,
1949, and Jaques, 1947, 1951). Usually mentioned was the very
close association between the beetle and its bracket fungus host,
its nocturnal behavior, and often a word about its havit of feign-
ing death when disturbed. Other workers have listed B. cornutus
as occurring, but not necessarily breeding, on a number of poly-
pores including, Pleurotus ostreatus, Polyporus tsugae, P.
lucidus, P. versicolor, P. perennis, Fomes applanatus, F.
fomentarius, and F. pinicola (Weiss, 1920 a,b, 1921, 1923, Weiss
and West, 1920a, 1921, and Liles, 1956).

This section is divided into five parts and the methods and
the important literature will be discussed separately for each part.

Mating Behavior

Many of my own observations on the beetles general habits
and mating behavior confirm what Liles (1956) reported. Therefore
this part is mainly a review of Liles' observations with only my
observations which add or contrast with his.

Methods

Aspects of the beetle's behavior including the recording of
the individual males and females in a "mounted" position were
noted during the day-time, bettle location checks. Nine night-
time observations, August 10, 12, 14, 16, 18, 24, 26, 31 and
September 2, were usually made after dark between 10:00 and
11:00 p.m.

Discussion of Literature and Results

B. cornutus generally were found resting during the day on
the under surface of sporocarps (sometimes on the top surface as
well, in an undisturbed situation), or in cracks and crevices in
and around the host fungus and adjacent bark on the tree or log
(Figs. 13 and 14). The beetles are active at night from 8:00 p.m.
to 4:00 a.m. (Liles, 1956), at which time the beetles feed and
perform their courting activities. The adult beetles appear to
feed on any part of the fungus except the actively sporulating
hymenial layer.

During the night, male and female beetles are commonly found
involved in an interesting mating behavior, first noticed by Park,
Lockett, and Myers (1931), but more completely described by Liles
(1956) as follows:

> Prior to the mating, the male beetle clasped the female
> in such a manner that the ventral surface of his abdomen
> rested on the dorsal surface of her thorax, and the
> ventral surface of his thorax rested on the dorsal sur-
> face of her abdomen. When in this position, the male
> rubbed the ventral surface of his abdomen across the
> two prominent tubercles which projected from the females
> thorax. This produced a distinct rasping sound audible
> at a distance of six to eight feet from the fungus.
> This noise making was carried on for one to two minute

Figure 13. Male (right) and female <u>Bolitotherus cornutus</u>
(X2) on sporocarp of <u>Fomes applanatus</u>. Female
is feigning death.

Figure 14. Male <u>Bolitotherus cornutus</u> (X4) on edge of
<u>Fomes applanatus</u> sporocarp.

periods interspersed by one or two minutes of quiet.
At the end of one of these periods of rasping, the
male reversed his position and copulated with the
female.

They further stated that the beetles also make this noise after

copulation and during the oviposition period, and they are often

found in the noise-making position with no noise being produced.

During the daytime pairs of beetles were commonly found, not

in the noise making position but reversed, in more of a copulatory

position; but they would not be in actual copula. Park et al.

(1931) also mentions seeing B. cornutus in this position from

11:00 p.m. to 1:00 a.m. In 1964, a total of 68 pairs were observed

in this position. The records of marked beetles show that an in-

dividual male or female beetle was often in a mounted position

with a different female or male on different occasions. For ex-

ample, three beetles were each observed in the mounted position

on three separate occasions and in every case the three had a

different partner. This observation suggests that the beetles

are polygamous. One pair (#38 male and #51 female) found in the

mounted position in 1965, had also been found in the mounted

position, each with a different beetle, in 1964 which indicates

that their reproductive activity may extend over two summer sea-

sons. Also, these two beetles and two others (#7 and #13) marked

in 1964 were found in the mounted position with beetles marked in

1965. By far, the most pairs in this position were observed from

the middle to the end of July with relatively few seen either

before or after this period.

Detailed observation of the beetles' behavior at night was nearly impossible because they proved to be very sensitive to any light used to aid in the observations. The direct light from an ordinary two cell flashlight caused the beetles to stop their normal activities and either feign death or crawl back into hiding. Both red and blue filters made to fit over the lens of the flashlight, greatly reducing the light, had the same effect. Also, it was found that the full moon did not afford enough light at the surface of the logs to observe the beetles. The significant exception to the usual disruption of the beetles' activities with artificial light was the oviposition process observed on August 16, with the unfiltered light from a flashlight. Once they had started the process the light had no apparent effect on them.

While this sensitivity prevented most prolonged observations, spot-checks of the beetle's position and their individual numbers could be noted. When the sporocarps were checked periodically on two evenings between 9:00 and 10:00 p.m. (CDT), when the area was becoming progressively darker, the majority of the beetles did not appear until it was almost completely dark. The beetles were commonly found crawling around on the upper surfaces of the sporocarps, but were also seen on the lower surfaces and on the bark of the log.

More beetles were observed at night than during the day especially from mid- to late-August 1964, when very few beetles were seen during the day. For example, only 13 and 9 beetles were seen on both the north and south logs during the day on August 16 and 18, respectively, while 32 and 25 were seen on the same dates

at night. As one would expect, the beetles were emerging from their hiding places to become active at night.

Observations Using Ultraviolet Light

Fluorescent markings have been successfully used to follow the movements of some beetles (Polivka, 1949 and Taft and Agee, 1962). It was thought that B. cornutus might be less affected by ultraviolet light so that observations of beetles marked with fluorescent paint would be possible at night.

Methods

A population of beetles on the dead aspen stump in site I which bore 10 to 12 sporocarps was used for this work. As many as possible of the beetles were individually marked with a fluorescent bulletin paint (Ultra-violet Products Inc., San Gabriel, California). Red and blue spots painted on the beetle's prothorax and elytra showed up well at night using a portable ultraviolet light (Blak-Ray light UVL 21, Ultra-violet Products Inc., San Gabriel, California) shown on them from a distance of 8 feet, 8 inches. Eighteen beetles were marked on June 13, 1965, and over the next three days six more were marked. The beetles were not checked during the day or handled more than necessary so they would be disturbed as little as possible. The beetles were observed using the ultraviolet light on the nights of June 13, 14, and 16. All times are Central Daylight Time.

Observations

The first night the light was turned on at 9:30 p.m. when the sky was still quite light, and the beetles were observed. By 10:00 p.m. when it was almost completely dark, the fluorescent spots showed well, but from this time until 10:35 p.m. the beetles appeared to move very little and nothing could be determined concerning their exact activities (i.e. whether they were feeding, courting, etc.). The following night the light was not turned on until after complete darkness (10:30 p.m.). The position of five of the beetles was carefully noted and 45 minutes later, two of the beetles had disappeared, one had moved slightly, and the other two had not moved. The third night the light was moved to about 18 feet from the beetles and turned on at 9:40 p.m. By 11:00 p.m. only three beetles were visible.

Observations using the ultraviolet light were then discontinued, because its use had not improved the ability to observe their behavior at night, and it also appeared to affect their behavior much as visible light had.

Observations in a Light-proof Hut

A nearly light-proof hut constructed over several sporocarps on the south log made it possible to determine the effect of almost constant darkness on the beetles' behavior.

Methods

The hut was 8 feet by 5 feet by 5 feet high on the front side and 4 feet high on the back side. The south log ran through the

middle of the hut perpendicular to the hut's long axis. Its

wooden frame was covered with 6 mil, black plastic which was cut

to fit around the long on each side of the hut. The roof was also

covered with aluminum foil to reflect the sun's rays and prevent

the temperature from getting too hot inside. The hut enclosed

five active, medium to large sized sporocarps on the front (North)

side and several smaller ones on the other side. The log was 19

inches in diameter at this point. The hut was completed on July 5,

1965. The beetles in the hut were observed during 14 days from

July 7, 1965, to August 23, 1965. The beetles' individual num-

bers, their general location, and any specific activities observed

were recorded. The light intensity inside the hut was judged to

be no more than that of a clear night with a half-moon. Neither

the beetles nor any details of the objects inside the hut could

be seen without the aid of a flashlight.

Continuous recordings of temperature (and relative humidity)

inside the hut were kept for comparison with the temperature

records kept at the Biological Station. The maximum temperature

in the hut averaged 2.6 degrees F. (range: 12° less to 1° more)

less, and the minimum temperature averaged 8.3 degrees F. (range:

20° more to 6° less) more than the maximum and minimum temperatures

at the Biological Station, respectively. Relative humidity inside

the hut during the day was generally 50 or 60 per cent except

during rainy weather when it was much higher. At night the rela-

tive humidity always went up to 90 to 100 per cent inside the hut.

Observations and Discussion

Park and Keller (1932), in their studies on nocturnal ecology, reared B. cornutus in the laboratory in continuous darkness with constant temperature and relative humidity. They found that B. cornutus retained their nocturnal habits, being active during night-time hours and inactive during the day-time hours. This was in contrast to the other nocturnal insects they tested which led them to conclude that B. cornutus has a "nocturnal periodicity, viz. a rhythm in the strict sense".

This conclusion is not consistent with my observations of beetle activity inside the light-proof hut. The beetles inside the hut would appear during the day very much as they normally appear at night outside the hut. During almost all of the checks inside the hut most of the beetles were on the upper surfaces of the sporocarps in what would ordinarily be plain view. On the rest of the log, outside the hut, rarely was a beetle seen in this situation during the day. Many more beetles were found inside the hut than could be found anywhere on the rest of the log. This is similar to the night situation when many more beetles are evident than during the day. For example, on July 9 and July 11, no beetles were found on the log during the daytime whereas six and five beetles, respectively, were found on the sporocarps inside the hut during these same daylight hours. Altogether only 31 beetle recoveries were made on the south log (exclusive of the part covered by the hut) in 10 daytime checks on 13 sporocarps from June 16 to August 23, 1965. This is in contrast to 54 beetle

recoveries made on the five sporocarps inside the hut in 14 day-time checks from July 7, 1965, to August 23, 1965. This may or may not have been caused by the beetles being out of their hiding places in the hut, as is typical during the night. Possibly the beetles were somehow attracted to the sporocarps inside the hut. However, the fact remains that the majority of beetles recovered in the hut were out in plain view rather than in cracks and crevices.

Besides commonly finding the beetles in typical "night-time positions", several beetles were observed engaged in activities usually done at night. On July 13, 1965, two beetles were found in copula, and on July 17, 1965, a female was observed in the process of oviposition.

While it can not be concluded from these observations that Park and Keller were incorrect, they do seem to indicate that the presence or the absence of light is a definite factor. Certainly much more work would have to be done on this problem before any conclusions could be made, especially since the entire concept of endogenous biological clocks is in question (Brown, 1960).

Defensive Behavior

B. cornutus is also well known for its habit of feigning death when disturbed. They typically draw in their legs close to the body and remain motionless, often rolling off the substrate to the ground in the process (Fig. 13).

Weiss (1947) determined the duration of B. cornutus death feints using two intensities of mechanical stimulation. He

disturbed the beetles by tapping them with a pencil or by blowing on them with a small bellows. At 77° F. the pencil tapping induced feints averaging 111-134 seconds long and the air puff induced feints averaging 17-18 seconds long. The amount of force in the stimulation thus, did effect the duration of the feint. The number of successive feints that could be induced varied from 23 to 40.

Besides the death feint, the beetles also exude a brownish, acrid-smelling fluid from two glands at the posterior end of the abdomen as part of their defensive behavior when handled. The glands were described by Auten (1933) as a pair of enlarged anal glands, yellow in color and oval in shape. They lie in front and above the rectum and consist of a mushroom-like cap of secretory cells anterior to a thin walled reservoir portion. Posteriorly the glands join to form a wide duct near the anus.

During the two summers of observing B. cornutus none were ever seen flying or making any attempt to fly, and the literature contains no record of this species flying (Graves, 1960). Also the beetles were never observed to lift their elytra to expose their hind wings. The elytra of these beetles are heavy and fit perfectly together when closed by means of a tongue and groove arrangement along their inner edges. The elytra of several live beetles were gently forced open, but it was difficult to do this without apparently injuring the beetle. Once the elytra were opened and the well developed hind wings unfolded, the beetles seemed to be unable, or at least they made no attempt to refold

the hind wings and elytra. Both male and female beetles have
large, well developed secondary flight muscles as well as the
usual complement of primary flight muscles.

Possibly, the beetles have lost the ability to fly because
of an inability to manipulate the heavy, armor-like elytra.

Movements of Adult Beetles

For Bolitotherus cornutus to disseminate spores of Fomes
applanatus, it must move to suitable inoculation sites, such as
wounds or broken branch stubs on weakened or recently killed
hardwoods. The movements of the marked beetles on the two large
aspen logs in site I were followed during the summer of 1964 and
during part of the succeeding summer. Periodic checks for sur-
viving marked beetles also were made in 1966.

Methods

A red enamel paint (brand name unknown) and a very fine
brush were used to mark the beetles. From one to five small spots
painted on nine standard areas on the prothorax and elytra in
varying combinations indicated the beetle's individual number.
The beetles on the south log were distinguished from those on the
north log by an additional spot painted on the posterior tip of
the elytra just above the anus. A blue paint was used in 1965
to distinguish the 1965 marked beetles from those marked in 1964.

Unmarked beetles were removed from the log or sporocarp,
held between the thumb and forefinger while being marked, and

then replaced as near as possible to their original position.
The fact that the beetles almost always feigned death after being
handled was helpful in allowing a moment for the paint to dry.
The paint appeared to be non-toxic to the beetles and was durable,
with the spots remaining plainly visible after two years. There
was no indication that it affected their behavior in any way.

On July 14, 1964, 25 beetles were located on the north log
and marked consecutively, and two days later the first 19 beetles
were marked on the south log. Subsequently, every two days (un-
less rain interferred, in which case the checks were made the
next day) the logs and sporocarps were carefully examined for
marked beetles, and any unmarked beetles found were marked. The
location of each beetle found was recorded using the numbered
sporocarps as reference points. The majority of the unmarked
beetles found were probably some of the native population which
had escaped notice during all the previous checks. However, newly
emerging adults from pupal cells within the sporocarps and pos-
sibly some immigrants from near by populations added to the number
of unmarked beetles.

The marking of new beetles was discontinued on August 20,
1964, after 132 beetles had been marked on the two logs. A total
of 26 day-time checks were made on the north log and one less was
made on the south log. The last check was on September 1, 1964.

Six night location checks were made starting on August 16,
1964, and ending on September 2, 1964. These were made after dark
usually between 10:00 p.m. and 10:45 p.m. A flashlight was used

to locate the beetles and to read their numbers. Because the primary concern was the location of as many beetles as could be found, the night and day data will be considered together, but a beetle seen twice in one day will be considered a single recapture.

In 1965 this work was continued during the first half of the summer with the marking of 24 new beetles. However, the population appeared to be much smaller than it had been the previous year, and the marking of new beetles was halted in early July, except for beetles which appeared in the observation hut. The movements of these beetles were not sufficiently different in any respect from the movements of the previous year's marked beetles to warrant detailed discussion. Therefore, location checks (mostly day-time), which had been made every other day during the first half of the summer, were then made intermittently during the second half mainly to locate as many of the beetles marked in 1964 as possible. The two logs were checked a total of fourteen times in 1965.

Results

The recapture data for each beetle in 1964 are listed in Tables 7 and 8. The recapture data for all the beetles marked in 1964 are summarized in Table 9. Of the 132 beetles marked on the north and on the south logs, 106, or 80 per cent of the beetles were recaptured during at least one of the 30 day or night checks between July 16 and September 2. Eighty per cent of the beetles

Table 7. Recaptures of B. cornutus marked on the north log in 1964. (fm = female, m = male, MP = mounted position)

Beetle no. and sex	Date marked	No. of recaptures 1964	No. of recaptures 1965	Date last seen 1964	Date last seen 1965	No. of recaps. at original location in 1964	No. of times seen in MP in 1964	Remarks
1 fm	VII-14	6	1	VIII-16	VIII-23	1	1	On log So. of So. log in '65
2 fm	VII-14	2	0	VIII-31	-	0	0	Moved to So. log in '64
3 m	VII-14	9	0	IX-2	-	5	1	
4 fm	VII-14	11	0	VIII-26	-	5	3	
5 fm	VII-14	5	0	VIII-26	-	4	0	
6 m	VII-14	12	1	IX-2	VIII-10	10	3	
7 fm	VII-14	3	0	VIII-2	-	1	0	
8 m	VII-14	0	0	-	-	0	0	
9 m	VII-14	2	0	VIII-31	-	2	0	
10 fm	VII-14	0	0	-	-	0	0	
11 m	VII-14	1	0	VII-16	-	0	0	
12 m	VII-14	5	0	VII-30	-	2	0	
13 fm	VII-14	3	0	VIII-16	-	0	0	
14 m	VII-14	0	0	-	-	0	0	

Table 7. Continued.

Beetle no. and sex	Date marked	No. of recaptures		Date last seen		No. of recaps. at original location in 1964	No. of times seen in MP in 1964	Remarks
		1964	1965	1964	1965			
15 m	VII-14	7	0	IX-2	-	6	2	
16 fm	VII-14	2	1	VII-20	VII-2	0	1	
17 fm	VII-14	6	0	VIII-4	-	0	2	
18 m	VII-14	19	0	VIII-26	-	18	0	
19 m	VII-14	5	0	VIII-31	-	0	0	
20 fm	VII-14	16	0	IX-2	-	13	2	
21 fm	VII-14	0	0	-	VI-12	0	0	
22 fm	VII-14	5	0	VIII-31	-	3	0	
23 fm	VII-14	6	0	VII-18	-	0	1	
24 m	VII-14	1	0	VII-19	-	1	1	
25 fm	VII-14	4	0	IX-2	-	0	0	
26 m	VII-16	10	0	IX-2	-	8	1	
27 m	VII-16	1	0	VII-28	-	0	0	
28 fm	VII-16	13	0	IX-2	-	0	3	

Table 7. Continued.

Beetle no. and sex	Date marked	No. of recaptures 1964	1965	Date last seen 1964	1965	No. of recaps. at original location in 1964	No. of times seen in MP in 1964	Remarks
29 m	VII-16	2	0	VIII-2	-	0	0	
30 fm	VII-16	3	0	VIII-18	-	0	1	
31 m	VII-16	0	0	-	-	0	0	
32 m	VII-16	2	0	VIII-26	-	0	0	
33 fm	VII-16	0	0	-	-	0	0	
34 fm	VII-16	8	0	IX-2	-	0	0	
35 m	VII-16	8	0	VIII-18	-	2	0	
36 m	VII-17	4	0	VII-23	-	3	3	
37 m	VII-17	1	0	VII-23	-	1	0	
38 m	VII-17	6	0	VIII-18	-	0	0	
39 fm	VII-17	2	0	VIII-31	-	2	0	
40 fm	VII-17	0	0	-	-	0	0	
41 m	VII-19	2	0	IX-2	-	1	2	
42 fm	VII-19	2	0	VIII-24	-	0	1	
43 fm	VII-19	4	0	IX-2	-	1	2	

Table 7. Continued.

Beetle no. and sex	Date marked	No. of recaptures 1964	No. of recaptures 1965	Date last seen 1964	Date last seen 1965	No. of recaps. at original location in 1964	No. of times seen in MP in 1964	Remarks
44 fm	VII-19	0	2	–	VIII-10	0	2	
45 fm	VII-19	1	0	VII-28	–	0	0	
46 fm	VII-20	1	0	VIII-2	–	1	0	
47 fm	VII-21	2	0	VIII-18	–	0	0	
48 fm	VII-21	6	0	VIII-26	–	1	0	
49 m	VII-21	6	0	IX-2	–	4	3	
50 m	VII-21	1	0	VIII-6	–	0	1	
51 fm	VII-22	15	1	IX-2	VII-2	1	1	
52 fm	VII-22	10	0	IX-2	–	10	1	
53 fm	VII-22	7	0	IX-2	–	7	1	
54 fm	VII-23	6	0	VIII-26	–	0	1	
55 fm	VII-23	4	0	VIII-6	–	0	0	
56 m	VII-26	1	0	VII-28	–	0	1	
57 m	VII-26	2	0	IX-2	–	0	1	
58 fm	VII-28	0	0	–	–	0	0	

Table 7. Continued.

Beetle no. and sex	Date marked	No. of recaptures		Date last seen		No. of recaps. at original location in 1964	No. of times seen in MP in 1964	Remarks
		1964	1965	1964	1965			
59 fm	VII-28	5	0	VIII-26	-	3	1	
60 m	VII-28	1	0	IX-2	-	0	0	
61 m	VII-30	0	1	-	VII-9	0	1	On So. log in '65
62 m	VII-30	4	0	VIII-26	-	0	1	
63 fm	VII-30	1	0	VIII-6	-	0	0	
64 m	VIII-4	4	1	IX-2	VI-16	0	0	
65 m	VIII-6	3	0	VIII-14	-	0	0	
66 m	VIII-10	1	0	IX-2	-	1	1	
67 fm	VIII-10	2	2	VIII-18	VIII-10	2	1	
68 m	VIII-10	0	1	-	VII-7	0	0	On So. log in '65
69 fm	VIII-10	5	1	IX-2	IX-1	0	0	
70 m	VIII-18	0	0	-	-	0	0	

Table 8. Recaptures of B. cornutus marked on the south log in 1964 including those recaptured in the observation hut. (fm = female, m = male, FB = fruit body, MP = mounted position)

Beetle no. and sex	Date marked	No. of recaptures		Date last seen		No. of recaps. at original location in 1964	No. of times seen in MP in 1964	Remarks
		1964	1965	1964	1965			
1 m	VII-16	6	0	VIII-24	-	5	1	
2 fm	VII-16	3	1	IX-2	VII-2	1	0	
3 m	VII-16	6	0	IX-2	-	0	0	
4 m	VII-16	4	0	VIII-26	-	1	0	
5 m	VII-16	2	5	VII-30	VII-11	0	2	
6 fm	VII-16	2	0	VII-28	-	1	2	
7 fm	VII-16	3	3	VIII-16	VII-27	0	0	
8 fm	VII-16	1	0	VII-23	-	0	0	Accidentally killed
9 m	VII-16	2	0	VII-24	-	0	0	
10 m	VII-16	1	0	VII-23	-	0	0	
11 fm	VII-16	4	0	VIII-6	-	1	0	
12 m	VII-16	1	0	VIII-16	-	0	0	
13 fm	VII-16	0	3	-	VIII-2	0	0	

Table 8. Continued.

Beetle no. and sex	Date marked	No. of recaptures 1964	No. of recaptures 1965	Date last seen 1964	Date last seen 1965	No. of recaps. at original location in 1964	No. of times seen in MP in 1964	Remarks
14 fm	VII-16	8	1	IX-2	VII-14	3	0	
15 fm	VII-16	1	0	VII-30	-	0	1	
16 fm	VII-16	8	0	VIII-31	-	0	2	
17 m	VII-16	5	0	VIII-4	-	0	1	
18 m	VII-16	2	0	IX-2	-	0	0	
19 m	VII-16	1	0	VII-26	-	0	0	
20 fm	VII-17	1	0	VII-28	-	0	0	
21 fm	VII-17	0	0	-	-	0	0	
22 fm	VII-17	8	2	IX-2	VII-11	0	1	On log So. of So. log in '65
23 m	VII-17	3	1	VIII-16	VII-15	1	2	
24 fm	VII-17	7	2	IX-2	VII-19	0	1	
25 m	VII-17	6	3	IX-2	VI-18	1	0	
26 fm	VII-19	3	0	VIII-16	-	0	0	
27 fm	VII-19	3	0	IX-2	-	0	0	

Table 8. Continued.

Beetle no. and sex	Date marked	No. of recaptures		Date last seen		No. of recaps. at original location in 1964	No. of times seen in MP in 1964	Remarks
		1964	1965	1964	1965			
28 m	VII-19	4	0	VII-24	-	0	0	
29 fm	VII-19	2	0	VIII-2	-	0	1	
30 m	VII-19	0	0	-	-	0	1	
30 fm	VII-20	2	0	IX-2	-	0	1	
31 fm	VII-21	2	0	VIII-4	-	0	0	
32 fm	VII-22	11	1	IX-2	IX-1	2	0	
33 fm	VII-22	4	0	IX-2	-	1	0	
34 fm	VII-22	0	0	-	-	0	0	
35 fm	VII-23	10	0	IX-2	-	1	0	
36 fm	VII-23	4	3	VIII-26	IX-1	0	0	
38 m	VII-26	3	7	VIII-6	VIII-23	1	1	
39 fm	VII-26	1	0	VIII-10	-	0	1	
40 fm	VII-26	0	1	-	VI-20	0	0	On log So. of So. log in '65
41 fm	VII-26	6	5	IX-2	IX-1	1	0	

Table 8. Continued.

Beetle no. and sex	Date marked	No. of recaptures		Date last seen		No. of recaps. at original location in 1964	No. of times seen in MP in 1964	Remarks
		1964	1965	1964	1965			
42 fm	VII-28	4	0	IX-2	-	0	1	
43 fm	VII-28	0	0	-	-	0	0	
44 m	VII-30	0	0	-	-	0	1	
45 fm	VII-30	2	0	VIII-10	-	0	1	
46 fm	VIII-2	0	0	-	-	0	0	
47 fm	VIII-2	0	0	-	-	0	0	
48 fm	VIII-4	2	2	IX-2	VII-9	0	0	
49 m	VIII-4	1	0	VIII-6	-	0	2	
50 m	VIII-4	0	0	-	-	0	0	
51 fm	VIII-6	2	6	VIII-26	VII-15	0	1	
52 m	VIII-6	2	2	VIII-31	VII-27	0	0	
53 m	VIII-6	1	0	IX-2	0	0	0	
54 fm	VIII-6	1	0	VIII-12	-	1	0	
55 m	VIII-6	2	0	IX-2	-	0	0	

Table 8. Continued.

Beetle no. and sex	Date marked	No. of recaptures		Date last seen		No. of recaps. at original location in 1964	No. of times seen in MP in 1964	Remarks
		1964	1965	1964	1965			
56 m	VIII-6	0	0	-	-	0	0	
57 m	VIII-10	3	0	IX-2	-	1	0	
58 m	VIII-10	2	0	IX-2	-	0	0	
59 m	VIII-10	2	0	VIII-18	-	0	0	
60 fm	VIII-20	0	0	-	-	0	0	

Table 9. Summary of the recaptures in 1964 and 1965 of <u>B. cornutus</u>
marked in 1964 on the north and south logs including
those recaptured in the observation hut.

	North log	South log	Total	Per cent of 132*
Total no. of location checks.				
Day-time 1964	24	23		
Night-time 1964	6	6		
Day-time 1965	11	11		
Night-time 1965	3	3		
Observation hut 1965		14		
Total no. of beetles marked.	71	61	132*	
Number of beetles recaptured at least once.	62	50	112	85
No. of beetles recaptured more than once.	48	39	87	66
No. of beetles recaptured five or more times.	26	16	42	32
No. of beetles recaptured at least once on the same sporocarp on which they were originally found (1964 recaptures only).	29	15	44	33
No. of beetles seen in the mounted position (1964 recaptures only).	29	19	48	36
No. of times pairs in the mounted position were observed (1964 recaptures only).	46	24	70	

	North log	South log	Total	Per cent of 60*
No. of beetles marked in the first three days.	35	25	60*	
No. of these recaptured after one month.	23	15	38	63
No. of these never recaptured.	5	1	6	10

recaptured were seen more than once and about one third were seen five or more times.

Most of the beetles which were recaptured fairly regularly were found on the same sporocarp, or on a sporocarp near the one they had been seen on last. Thus, a movement of more than a few feet in a 48 hour period was uncommon. Beetles number 22 on the south log and numbers 52 and 35 on the north log were such exceptions; all having moved at least 15 feet in a 48 hour period, with the first beetle doing this on two occasions. At the other extreme were five beetles on the north log (numbers 6, 18, 20, 26, and 52) which were found on the same sporocarp at least eight times. Number 18 was found in the exact same spot on the same sporocarp 18 times.

Beetles not seen for a period of a week or more were much more likely to be found a greater distance from their last known position. However, only seven beetles on the north log and 10 on the south log moved from near one end of the log to the other some time during the seven weeks that checks were made. Only one beetle was recorded moving from the north log to the south log in 1964 and none were found to have moved in the opposite direction.

During the first three days (July 14, 16, and 17) 60 beetles were found and marked on the two logs. The data on these beetles in particular was checked to determine the number still being recaptured one month after they had been marked. The purpose of this was to check the possibility that the beetles were being recovered only soon after they had been marked, and were

subsequently moving out of the area.

Thirty-eight of these beetles (63%), including beetles re-captured in 1965, were recaptured at least once after August 15, 1964 (see Table 9). Therefore, nearly two-thirds of the original 60 beetles could be accounted for after one month. This is inde-pendent of the fact that most of these beetles were also recap-tured at least once before August 15, 1964. Only six beetles in this group were never recaptured either in 1964 or in 1965.

In 1965 27 of the beetles marked in 1964 were recaptured at least once (see Tables 7 and 8) during the 14 checks on the two logs or during the 14 checks on the sporocarps in the observation hut. Six of these beetles had not been recaptured in 1964 which means that only 20 of the 132 beetles marked in 1964 were never recaptured. Two beetles marked on the north log and moved to the south log and three other beetles were found on the log 20 feet south of the south log. Two of these were from the south log and one was from the north log. Only 24 new beetles were marked in 1965 and, in general fewer beetles were seen on the two logs than in 1964.

Discussion

The data indicate that B. cornutus generally does not move from the vicinity of the host fungus and whatever movements are made, are to other sites of existing sporocarps.

The 22 beetles of the first 60 marked not recovered after one month are considered not a large enough proportion to indicate

a general movement of the beetles away from the area of the host

fungus. Natural mortality would certainly eliminate some of the

beetles, although there is no way to determine from these data

how significant this was. Also, the beetles in their natural

habitat have a multitude of places to hide from the observer.

This became apparent in late August when relatively few beetles

were seen during the day-time checks. The fact that many beetles

were still in the area was revealed by the night-time checks during

this period. One hundred, fifty-six (82 on the north log, 74 on

the south log) beetle recoveries were made during six night checks

between August 16 and September 2, while only 45 (34 on the north

log, 11 on the south log) beetle recoveries were made during seven

day-time checks in the same period.

Two factors probably contributed to the scarcity of the

beetles during the day. Handling the beetles every other day to

determine their numbers seemed to make them more "wary" than nor-

mal. At the start of this work many of the beetles could be found

on lower surfaces and occasionally even the upper surfaces of the

sporocarps. By mid-August almost none were found in these situa-

tions. On some checks the only beetles found at all were those

teased out of known hiding places. The second factor may have

been the unusually wet and cold weather which prevailed in late

August, 1964.

Not unexpectedly the beetles appeared to move to existing

healthy sporocarps when they did move. Wood decay fungi often

grow for several years in the wood before a sporocarp is produced.

Thus, B. cornutus, which is dependent on existing sporocarps during every stage of its life cycle is not likely to be attracted to areas where they do not already exist. The trend in the movements of the few beetles that were found moving from log to log was from the north log to the south log and to the log 20 feet south of the latter (although one beetle marked in 1965 did move from the south log to the north log). This appears to be consistent with the idea that they are attracted to healthy sporocarps, because of the effect of extensive flooding in the area in the spring and early summer of 1965. Throughout most of June the water level in the depression was about half way up the height of the logs which flooded and killed many of the lower sporocarps on both logs. On June 26, the water was pumped out to a level below the logs, but the deterioration of the lower sporocarps made them unsuitable for B. cornutus. The south log had been less affected than the north log and the log south of the south log had not been flooded at all. Therefore, the presence of more healthy sporocarps is probably what attracted the beetles to the south logs.

The flood was probably also important in reducing the overall population from the 1964 level. Besides reducing the number of suitable feeding and breeding sites, any larvae or pupae inside the flooded sporocarps must have been killed. Also beetles dropping from the substrate, as they often do when feigning death, would land in the water and may have been lost.

In conclusion the data from the marked beetles and the observations of their behavior indicate that <u>B</u>. <u>cornutus</u> remains closely associated with <u>F</u>. <u>applanatus</u> and moves only to sites of existing sporocarps. On the other hand there was no indication that this beetle moves to, or comes in contact with suitable inoculation sites where the fungus does not already exist. Thus, it is unlikely that <u>B</u>. <u>cornutus</u> is a "direct" disseminator of its host fungus.

Apart from the dissemination question, the data from the marked beetles have shown that the <u>B</u>. <u>cornutus</u> adults are fairly long lived. The 25 1964 marked beetles recovered in 1965 represent a group of adults of various ages, all of which successfully overwintered. Nineteen of these beetles were marked in July of 1964 and 11 were at least a year old when last seen.

This may indicate that the two distinct broods described by Liles (1956) overlap considerably. If his life cycle diagram (page 334) is interpreted literally, the adult beetles which will overwinter do not emerge until almost the middle of September, and the first brood of adults (from overwintering larvae) emerge in early July and die sometime in October. Since no beetles were marked after August 20, theoretically none of the marked beetles should have overwintered. Liles' work was done in Northern Michigan where the climatic conditions and seasonal lengths would be generally comparable to those of the Itasca area.

The main exceptions to Liles' two brood annual cycle appear to be the extended life span of the adult beetles, and, as mentioned before, the possibility of reproductive activity extending

over two summers. Besides the 11 beetles found in 1965 which

were over one year old, several beetles were found in the summer

of 1966 by two biologists familiar with the marked beetles (Dr.

French and Mike Ewert). One of these beetles (numer 64, a male

marked August 4, 1964) was collected alive, and I was able to

confirm that it was at least twenty-six months old when last

seen. Another beetle (number 46, male) from the aspen stump in

site I, marked on July 6, 1965, was found by the author near this

stump on June 10, 1967, making the beetle at least twenty-three

months old. It is possible that B. cornutus can survive more

than two years in the field since it is known that some beetles

and tenebrionids especially can live up to seven years (Rockstein,

1956).

Dispersal and Colonization Experiments

An important question unanswered by the data from the marked

beetles on the north and on the south logs is how the beetles move

to or colonize new locations of a host fungus. Nothing has been

mentioned in the literature concerning the dispersal of B.

cornutus, but they must be able to get from one location of sporo-

carps to another. Two dispersal experiments were set up using

marked beetles and transported sections of logs bearing sporocarps.

The two experiments will be discussed separately.

Methods: First Experiment

The population of beetles on the aspen stump in site I used

for the ultraviolet light work was used for the first dispersal experiment. The sporocarps on the stump were grouped in a clump 3 to 4 feet from the ground. Only two of the 10 to 12 sporocarps were in good condition with live, current-year hymenial layers covering the entire bottom of the sporocarps. The others were in various stages of deterioration from those with a partial current-year hymenium (poor) to those which were completely dead (dead). The stump was 150 feet south of the south log and 120 feet from the nearest naturally occurring F. applanatus sporocarps.

As described before, the beetles on the stump were marked with fluorescent paint using a two color marking system. Thirty beetles were marked from June 13 to June 22, and an additional 8 beetles were marked over the next month. On June 23, 4 sections, which included one or more sporocarps, were cut from aspen logs elsewhere in the area. The log sections plus a small birch stump bearing 3 sporocarps were transported and placed around the aspen stump at varying distances. The imported sporocarps, all F. applanatus, were carefully inspected for the presence of any in-digenous beetles, and those found were removed. The condition of the sporocarps borne by the sections ranged from good, to poor, to dead. This provided the beetles with a choice to determine whether they might choose one type predominantly over another.

The log sections were numbered and placed around the aspen stump (source stump) as follows: Log number one was placed 7 feet (measurements are from closest sporocarp on source stump to closest sporocarp on the log) south of the source stump. It bore a large

sporocarp in good condition which was divided into interconnect-
ing upper (9 inch by 3 inch) and lower (9 inch by 3 inch) parts.
Log number two, which bore one sporocarp in poor condition ($6\frac{1}{2}$
inches by 4 inches) was placed 7 feet, 4 inches west of the source
stump. Log number three, with one apparently dead sporocarp, was
placed 6 feet, 3 inches northeast of the source stump. The birch
stump, number four, was placed 13 feet, 6 inches southeast of the
source stump. It bore three sporocarps, the lowest ($6\frac{1}{2}$ inches by
$4\frac{1}{2}$ inches) was in good condition, the middle (4 inches by 3 inches)
one was in fairly good condition with the new hymenial layer not
quite covering the lower surface, and the top one (3 3/4 inches
by $2\frac{1}{2}$ inches) was dead. Log number five had two sporocarps in
good condition with one (4 inches by 2 3/4 inches) above the other
($5\frac{1}{2}$ inches by 1 inch) and was placed 22 feet, 2 inches southeast
of the source stump.

Thus, the first three logs were arranged relatively close to
the source stump, forming a triangle around it. The birch stump
and log number five were further away, more or less in line with
log number one. The introduced sporocarps were then checked over
the next month for the appearance of the marked beetles from the
source stump, and at the same time the sporocarps of the source
stump were checked to see which beetles remained there.

Results

The locations of each beetle found on the dates the logs were
checked are shown in Table 10. The first check was made on June
26 at 5:00 p.m. (CDT) three days after the logs were introduced.

Table 10. Location of marked B. cornutus found during each check in the first dispersal experiment. (L1,2,3,5 = Log 1,2,3,5 respectively; S4 = Stump 4; SS = Source stump)

Beetle no. & sex	June 26	28	July 2	4	6	9	11	13	15	17	19	23	25	27	29	31	Aug. 2
1 fm																	
2 m													SS*				
3 fm	L5	L5											SS				L1*
4 fm				SS		SS											
5 m													SS				
6 m						SS											
7 m				SS							SS						
8 m																	
12 m				SS													
13 m							L1		L5								
14 fm																	
15 fm																	
16 fm				S4	L1*								S4				
17 m	L1					L2											
18 fm	L1		L3	L1			L1		L1								
21 fm				SS					SS								
23 fm	L1											SS					
24 fm	L1																
25 m	L1	L1		S4												S4	
26 m																	
27 fm						SS											
28 m		L1															
31 m				L1	L1			L1	L1	L1						L1	
32 fm																	
34 fm				SS		SS					SS	SS					
35 m	L1			SS								SS					
36 fm	L1			S4					L1		L1	L1				L1	
36 fm				L1	L1*				L2								
37 fm											SS	SS					L1
38 m				S4	L5	L5	L5	L5	L5				L5	L5	L5	L5	L5

Beetles marked (M) on the source stump after June 26, 1965.

Beetle no. & sex	June 26	28	July 2	4	6	9	11	13	15	17	19	23	25	27	29	31	Aug. 2
41 m		M	SS							SS							L1*
42 fm		M															
43 fm			M														
45 fm				M									SS*	L1			
46 m					M							SS					
51 fm												M					
52 fm												M					
53 m												M					

*Beetles found in the mounted position.

By this time seven beetles were already on log number one, 7 feet from the source stump, and one beetle was on log number five, over 22 feet from the source stump. The second check two days later revealed only two of the original seven beetles on log number one, but the same beetle was on log number five. By July 2, one beetle had been found on log number three, and by July 4, four were found on the birch stump. The first beetle did not appear on log number two until July 9.

Of the three logs within about 7 feet of the source stump, number one was by far the most attractive to the beetles (Table 11).

Table 11. Number of B. cornutus found on each introduced log in the first dispersal experiment.

Introduced log	No. of marked beetles found on each	No. of unmarked beetles found on each	
		through July 4	after July 4
Log # 1	14	2	2
Log # 2	2	8	1
Log # 3	1	1	
Stump # 4	4		
Log # 5	3	6	
Remained on Source stump	9		
Returned to Source stump	4		

Six beetles of the first 30 marked never recovered.

Fourteen beetles of the original 29 marked before June 23 appeared on this log between June 26 and August 4, compared to only two found on log number two and one found on log number three (which

later moved to log number one) during the same period. Nine beetles recorded on the source stump were not found on any of the introduced logs during the experiment.

Some beetles showed rather extensive and unexpected movements. For example, numbers 23 (female) and 35 (male) both moved to log number one by June 20, but then returned to the source stump in July. Number three (female) moved to log number five, 22 feet from the source stump within three days, then on July 25, was found back on the source stump (in mounted position with number 45 (male)) and on August 2, was recorded on log number one (in mounted position with number 41 (male)). Three beetles moved outward to the birch stump or log number five via one of the logs closer to the source stump.

Discussion

The movements of these beetles show that they do readily colonize new sources of a host fungus and can move at least 22 feet in three days. This work also supports the idea that the beetles are attracted to existing sporocarps, and apparently they prefer healthy, active sporocarps. Log number one was heavily favored, and, while farther away, the birch stump and log number five attracted more beetles than either log number two or three. Also, the beetles found on the latter two logs did not remain there long.

One complicating factor was the number of unmarked beetles which appeared repeatedly on the introduced logs, especially on log number two (Table 11). These beetles were removed when found.

A total of 17 were found on the four logs between June 28 and
July 4, and only three more were found after July 4. This sug-
gests that they came with the introduced logs either as new adults
emerging from inside the sporocarp for the first time, or as older
adults, simply well concealed in cracks and crevices. The fact
that eight unmarked beetles were found on log number two in 11
days while only two marked beetles were found there in 42 days
also indicates the introduction of some beetles with the logs.
However, some of these could have been beetles from the source
stump which escaped being marked, as undoubtedly some did.

A third possibility is that they were beetles from outside
the experimental area which means, assuming they came directly
from existing sporocarps, they moved at least 120 feet. This
possibility certainly can not be discarded because of the distance
involved as shown by the fact that on July 13 beetle number 10
(marked in 1964) from the south log was found on introduced log
number 5. This represents a move of about 175 feet by this
beetle which, before this, was last seen on July 23, 1964, on
the south log.

A second dispersal experiment was carried out in site IV in
order to observe the movements of the beetles more closely and to
avoid some of the problems encountered in the first experiment.
Also, by introducing a known number of beetles a better idea could
be obtained about what portion of the population tended to move
early, late, or not at all. Site IV was picked because of its
openness and because no sporocarps of Fomes applanatus could be

found in the area. Thus it was assumed that no B. cornutus were present.

<u>Methods: Second Experiment</u>

Twenty-three marked beetles and five sections of logs bearing sporocarps of F. applanatus were introduced into site IV. The five logs were arranged in a small clearing in a pattern very much like that used in the first experiment. Most of the low vegetation in the clearing was cut or trampled down so that observations could be made more easily (Fig. 15). Log number five from the first experiment which bore two sporocarps was used as log number one. All the other log sections were freshly cut from aspen logs in site I and each bore a single sporocarp. All the sporocarps were alive and actively sporulating when introduced on August 7, 1965. However, two of these appeared to be dead by August 26, probably because the log sections became too dry due to exposure to direct sunlight in the clearing and the small size of the sections themselves.

The release point for the beetles was a 4 inch diameter circle centered on top of a log which bore no sporocarps. Introduced logs one, two, and three were $6\frac{1}{2}$ feet northwest; 6 feet, 2 inches southwest; and 6 feet, 2 inches east of the release point respectively. Logs four and five were $14\frac{1}{2}$ feet and 22 feet east of the release point respectively. (All measurements are from the release point to the closest edge of the sporocarp on each log.)

The beetles used were the unmarked beetles collected over a period of weeks from the logs in the first experiment. A total

of 19 were collected, kept in the laboratory and marked from one
to 25 using the two color marking system. Three beetles collected
in site I, plus number 38 from the first experiment were added
making a total of 33 beetles. The beetles were released on the
release point at 1:45 p.m. (All times are CDT) on August 7, 1965.
The weather that day was generally warm, sunny, and humid with a
light wind from the south. The beetles were observed until 7:45
p.m.

Figure 15. Arrangement of the introduced logs in the
second dispersal and colonization experiment.
Picture was taken facing west with log number
five in the foreground and the releasing log
in the background (with field notebook leaning
against it).

of 19 were collected, kept in the laboratory and marked from one to 25 using the two color marking system. Three beetles collected in site I, plus number 38 from the first experiment were added making a total of 23 beetles. The beetles were released on the release point at 1:45 p.m. (all times are CDT) on August 7, 1965. The weather that day was generally warm, sunny, and humid with a light wind from the east. The beetles were observed until 7:45 p.m. They were checked four times during the next two days and once a day after that.

In a final experiment, to determine whether the beetles would readily return to the sporocarps if removed and released at the release point for a second time, 14 beetles found during the checks between August 18 and 23, including three unmarked beetles, were collected and saved. These beetles were released on August 23 at 5:15 p.m.

Results

For 6 hours after being released the beetles appeared to make little direct attempt to locate a sporocarp.

During the first 4 minutes the beetles recovered from their death feints and walked down the sides of the releasing log. After 10 minutes most of the beetles had come to rest mainly on the north or shaded side of the log and here they remained motionless for several hours. Gradually some of the beetles began moving again, and by 7:45 p.m. many had disappeared from view. Three were found on the ground $5\frac{1}{2}$ hours after being released, but they were not moving. Number five was 15 inches southwest of the

releasing log or in the direction of log number two. This beetle
first appeared on log number three however, and remained there
consistently. Beetles number 13 and 24 were found a few inches
north of the releasing log. Number 13 was never recovered again,
but number 24 was later found on log number three. The introduced
logs were checked periodically during the first 6 hours for the
appearance of any beetles but none were found.

The locations of the beetles found during each of the subse-
quest checks is shown in Table 12. The first check on August 8
was made at 9:00 a.m. and one beetle (number three) was found on
log number one. Thus, within $16\frac{1}{4}$ hours this beetle was able to
find a sporocarp $6\frac{1}{2}$ feet away. No other beetles were visible,
either on the other introduced logs, the releasing log, or on
the ground. On August 9 the second beetle to appear was found
on log number three. The first and only beetle (number 27) to be
found on log number five, 22 feet away was found on August 10,
three days after being released. On the subsequent daily checks
all the beetles except four appeared at least once on one or more
of the introduced logs. The data are summarized in Table 13.

Of the 14 beetles which were collected and released again on
August 23, only three were found on the subsequent daily checks
through September 1. However, one of these (number five), a con-
sistent resident of log number three, had moved back to the same
log by 7:45 a.m. on August 24, or within $14\frac{1}{2}$ hours. The other
two had been unmarked beetles, both of which went to log number
one.

Table 12. Location of marked B. cornutus found during each check in the second dispersal experiment. (L1,2,3,4,5 = Log 1,2,3,4,5 respectively)

Beetle no. & sex	8	8	9	9	10	11	12	13	14	15	16	17	18	19	20	21	23
1 m							L1				L1	L1		L1 removed			
2 fm						L2	L2	L2	L2		L2	L2	L2 removed				
3 fm	L1						L1*		L1				L1 removed				
4 fm								L1	L1								
5 fm			L3	L3	L3	L3		L3	L3	L3	L3	L3	L3 removed				
6														1L removed			
7 m						L1	L1*		L1								L1 rem.
8																	
12 fm									L1					L1 removed			
13 fm																	
14 m									L4		L4	L4					L4 rem.
15																	
16 m									L1								L1 rem.
17												L2					
18 m						L3		L3	L3			L3*	L3 removed				
21 fm								L3*		L3		L3*					
23									L3		L3			L3 removed			
24 m							L3		L3								
25																	
26									L2								
27 fm					L5		L5			L1							L2
28 fm						L3											
38 m					L3	L3		L3*	L3								

Beetles released for the second time on Aug. 23	24	24	25	26	27	28	31	1
1								
2								
3								
5		L3	L3					
6								
7								
12								
14								
16								
18								
23								
31						L1	L1	L1
32								
34					L1		L1	L1	L1

*Beetles found in the mounted position

Table 13. Number of B. cornutus found on each introduced log in the second dispersal experiment.

Introduced log	No. of marked beetles found on each	No. of unmarked beetles found on each
Log # 1	8	1
Log # 2	3	3
Log # 3	7	1
Log # 4	1	1
Log # 5	1 (went to log # 1)	2

Four beetles never recovered.

Discussion

Basically, the results of both the first and the second dispersal experiments were similar, but one must be cautious in making comparisons because of the differences in the conditions involved. In both cases one beetle had found the sporocarp farthest from the source, and at least two had found a sporocarp closer to the source within three days. Also it took at least four to five days for the majority of the beetles to move to the introduced sporocarps. This was generally true for both experiments, but most clearly demonstrated in the second where the beetles were released on a log which bore no sporocarps.

The fact that the beetles are capable of moving 22 feet in two days while the majority appeared to move only 7 feet in four to five days suggests two possible modes of movement. One is that the beetles move randomly and locate the sporocarps by chance, thus taking most of the beetles a few days to find the fungi.

The second is that the beetles are attracted directly to the sporocarps but tend to remain on the releasing log (or source stump) for a few days before moving. The latter seems to be the most likely possibility since the beetles are so closely associated with and dependent upon the fungi. The fact that the beetles in the first experiment moved from one log to another fairly readily lends support to the second possibility. Possibly the sense of smell plays a part in their location of the sporocarps but specific experiments would have to be set up to test this.

The main difference in the results of the two experiment was the lack of movement by the beetles in the second experiment after they had found a sporocarp. Only one beetle is recorded to have moved from one sporocarp to another in the second experiment. Also, only one beetle was recorded on each of the two logs outside the inner triangle of logs. This may have been caused by the dryer and more open location in site IV. This seemingly less favorable environment may have discouraged further movement once the beetles located a sporocarp.

The careful observations made during the second experiment failed to shed much more light on the exact manner in which the beetles sought out the host fungus or how they were attracted to it. As one would expect, and as the observations on the day the beetles were released indicate, the beetles most likely moved at night. As far as could be determined all movements were by walking.

Surprisingly, a total of eight unmarked beetles were found on the sporocarps during the second experiment. It is assumed that they were all introduced, unnoticed along with the log sections.

In conclusion, the two experiments showed that B. cornutus: (1) readily seeks out new sources of host fungi, either from declining sporocarps or from a log without sporocarps, (2) can move at least 22 feet in three days, (3) prefers moist, live sporocarps, and (4), in the natural environment of the first experiment, are not always "content" to remain on the same sporocarp whether it is in good or in poor condition.

SUMMARY

The possibility of insect dissemination, either by random
or accidental movements of arthropods in general, or by direct
movements of a specific insect has been investigated.

Experiments on random dissemination included trapping in-
sects attracted to Fomes igniarius sporocarps and artificial
wounds in an over mature aspen stand, isolating possible wood
decay fungi from a sample of the trapped insects, and a test of
the ability of a variety of arthropods to carry spores and deposit
them on artificial wounds. The partial success of these experi-
ments allows one to conclude that it seems likely that some in-
sects would visit both wounds and sporocarps in their life span,
and that arthropods can carry spores on their bodies and deposit
them on wounds. A wood decay fungus was isolated from a few
insects but it is still not known whether enough insects in
general carry enough inoculum to establish a wood decay fungus
at a new site. Improved experimental planning and techniques,
especially a reliable method of isolating wood decay fungi, would
probably have given more conclusive results.

The common forked fungus beetle Bolitotherus cornutus was
investigated as a possible direct disseminator of its host fungus,
which is mainly F. applanatus in the Itasca area. Observations
of the beetles' behavior and movements indicate that it remains
close to the sporocarps of its host fungus and if it does move,
it is to sites of existing sporocarps. Colonization experiments

showed that this beetle will readily move 22 feet or in some cases much more in seeking out new sporocarps, apparently without the aid of flight. It was concluded that this insect was probably not a direct disseminator of _F. applanatus._

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