STUDIES ON INSECT DISSEMINATION OF WOOD ROTTING FUNGI

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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readily survive the air-borne journey and can become successfully established upon arrival (French and Christensen, unpublished).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 separately 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 carrying the fungus, and that it could be inoculating the trees either during the chewing of the egg notch by the adult or during oviposition.

Other insects, while not actually disseminating wood rotting fungi have been shown to be important factors in allowing the fungi 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 associated 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 dissemination of wood rotters is scanty, a number of papers have been published 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 dissemination 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 replaced the overmature aspen and large red pines in the area. The area contained several large aspen logs bearing numerous sporocarps 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 1½ to 12 inches and is 1½ 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 1½ to 8 inches wide and they project out ½ 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

Figure 1. Sporocarp trap, site II.

Figure 2. Wound trap, site II.

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 effectiveness 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 sporocarps 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 collection one day. The insects were removed and put in dry vials labeled with the tree number. The insects removed from the sporocarp 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 determined to order. The groups collected plus the number of individuals 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 relatively 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 number of times the insect and arthropod groups (plus the number of individuals in these groups) appeared in each of the four sets of

Figure 3. Control trap, site II.

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

1964				1965		
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, excluding 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	4	6	24	8	10	90
Site III				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 computed 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 similarity 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		No. of Individ. Collected		% of Times Collected		% of Individ. Collected	
	1964	1965	1964	1965	1964	1965	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	8.9	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		No. of Individ. Collected		% of Times Collected		% of Individ. Collected	
	1964	1965	1964	1965	1964	1965	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		No. of Individ. Collected		% of Times Collected		% of Individ. Collected	
	1964	1965	1964	1965	1964	1965	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).

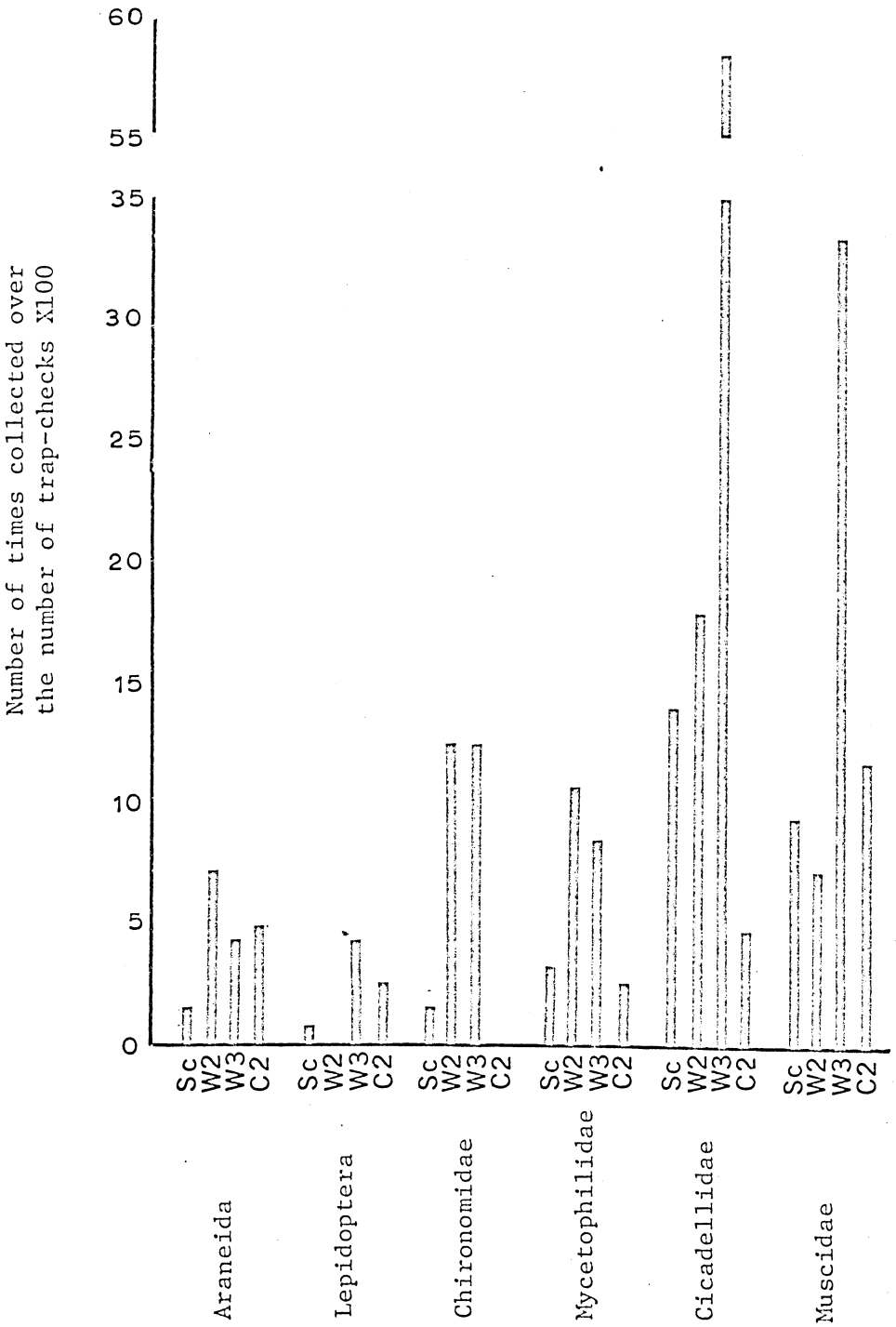


Figure 5. Per cent of times common groups were collected per trap per check in each set of traps in 1965. (Sc= Sporocarp traps; W2=Wound traps, site II; W3= Wound traps, site III; C2=Control traps, site II).

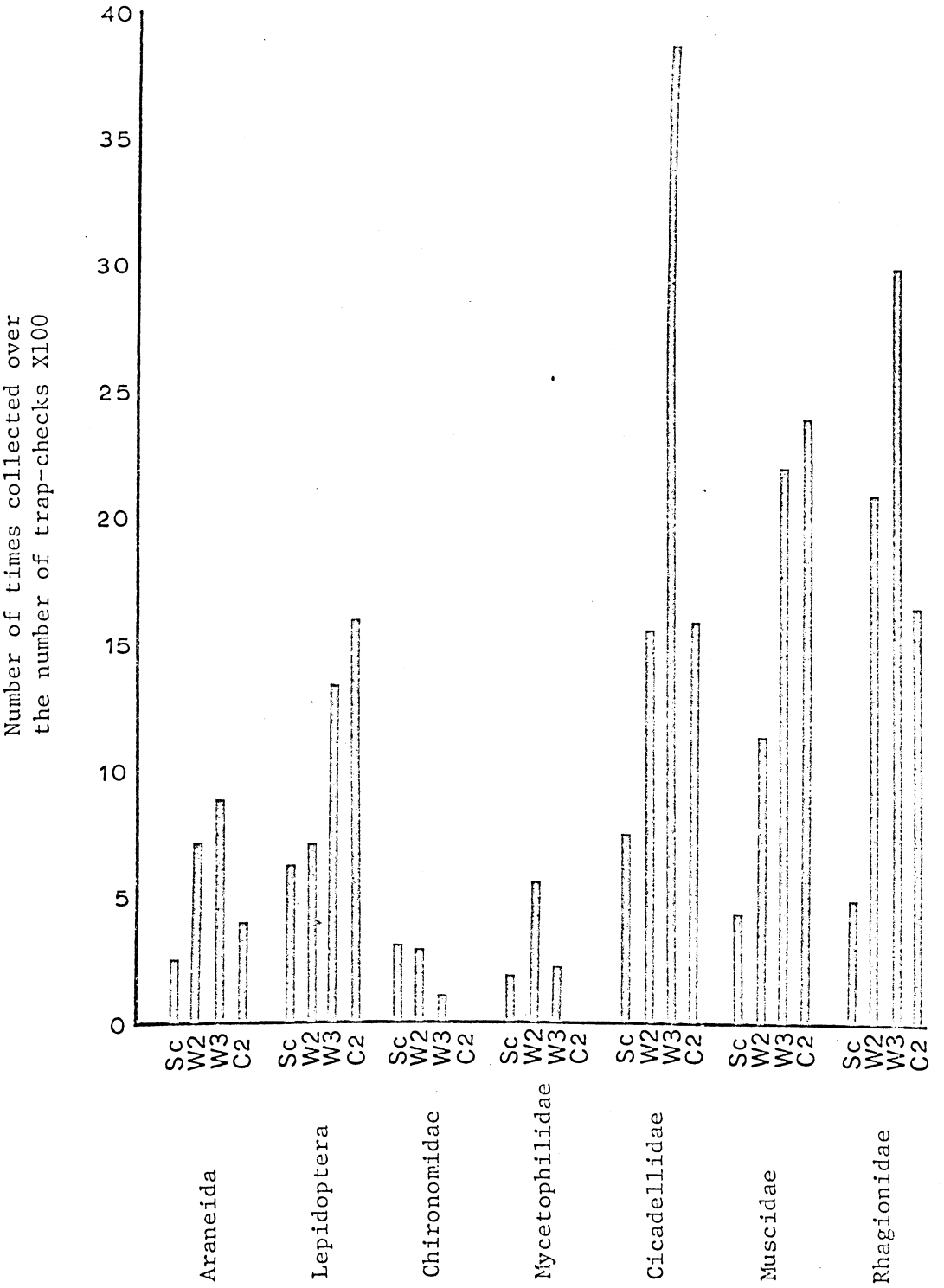


Figure 6. Per cent of individuals in the common groups collected per trap per check in each set of traps in 1964. (Sc=Sporocarp traps; W2=Wound traps, site II; W3=Wound traps, site III; C2= Control traps, site II).

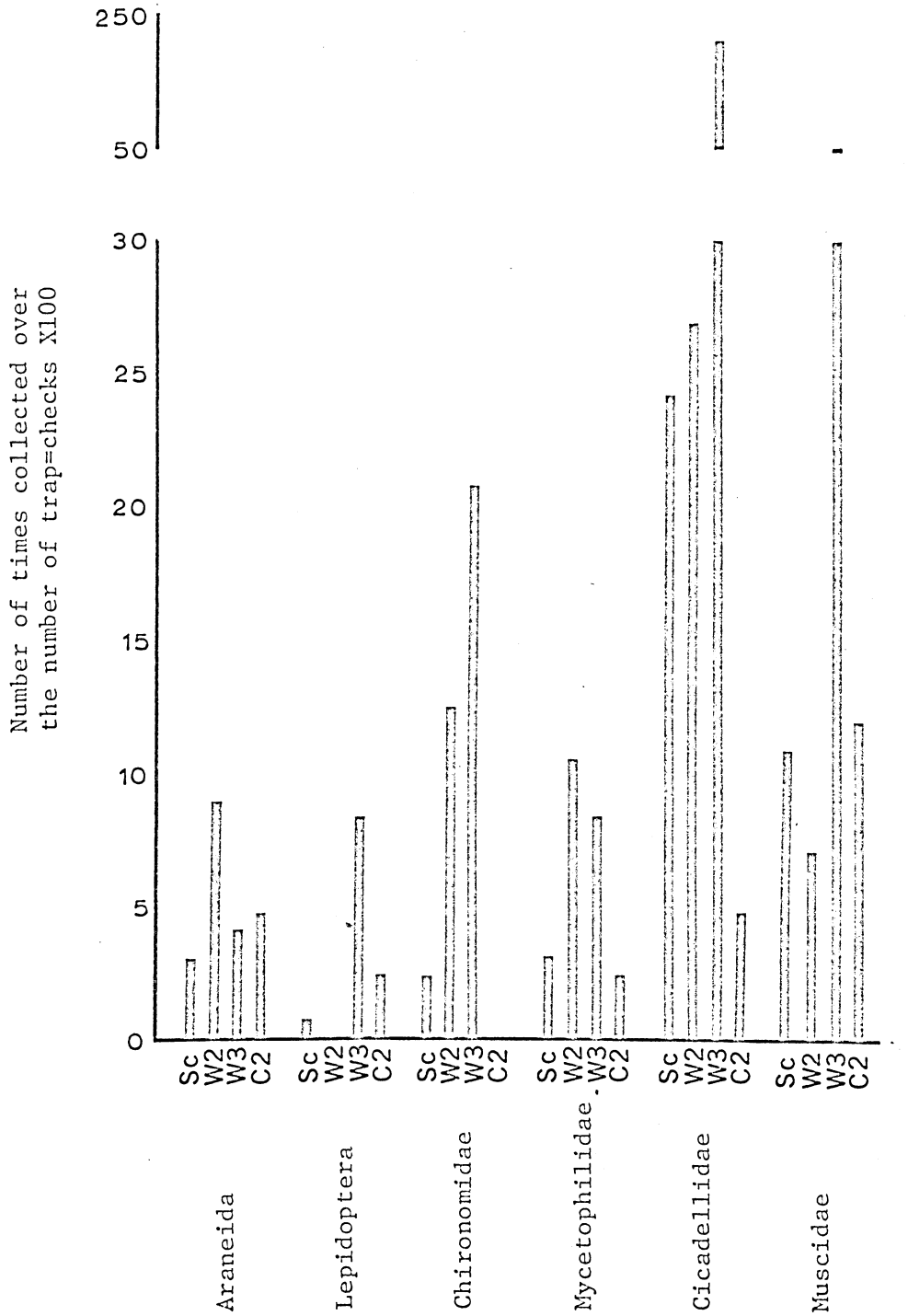


Figure 7. Per cent of individuals in the common groups collected per trap per check in each set of traps in 1965. (Sc=Sporocarp traps; W2=Wound traps, site II; W3=Wound traps, site III; C2= Control traps, site II).

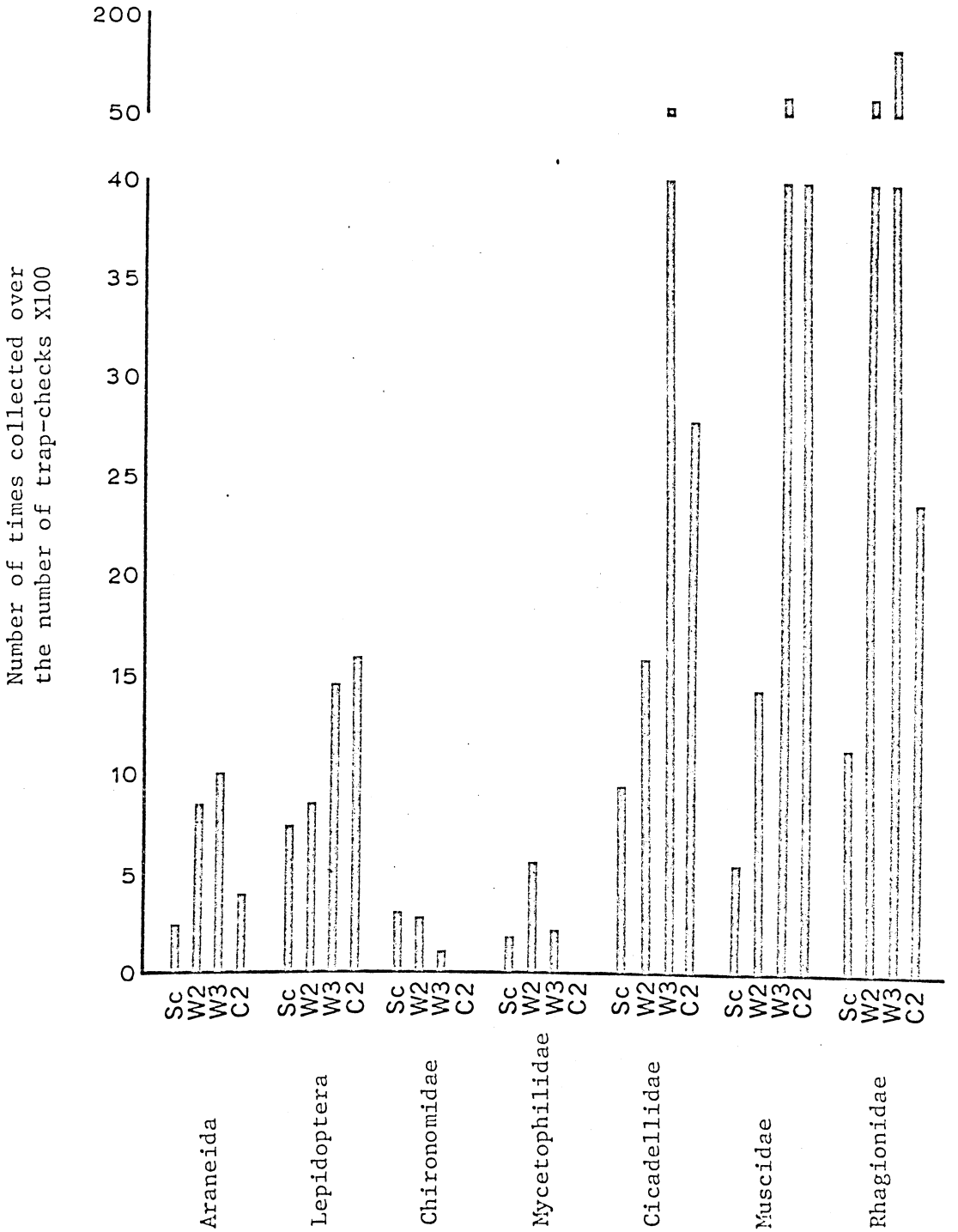


Figure 8. Per cent of times each common group appeared in two sets of traps in 1964. (Sc=Sporocarp traps; W2=Wound traps, site II; W3=Wound traps, site III; C2=Control traps, site II).

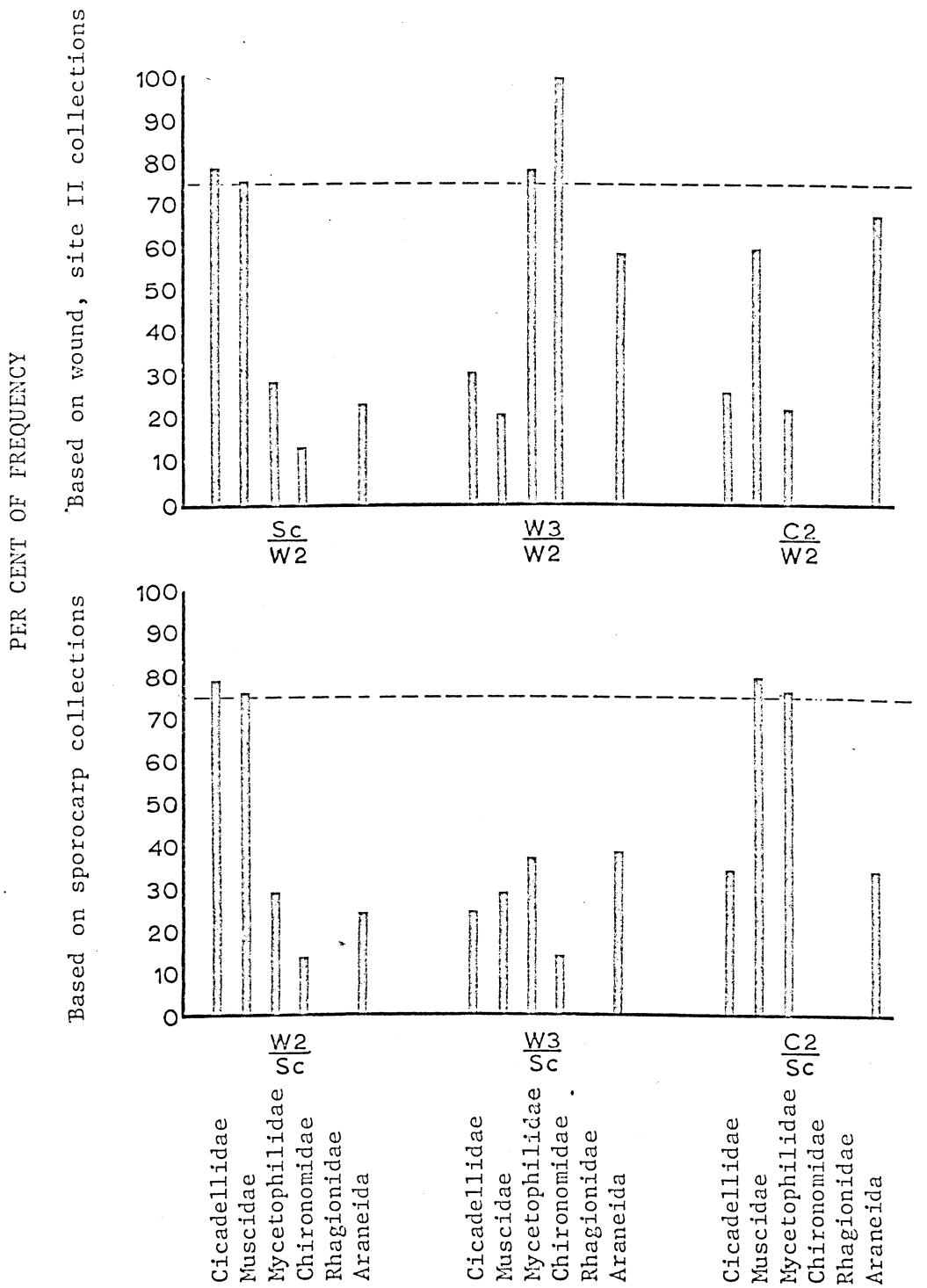
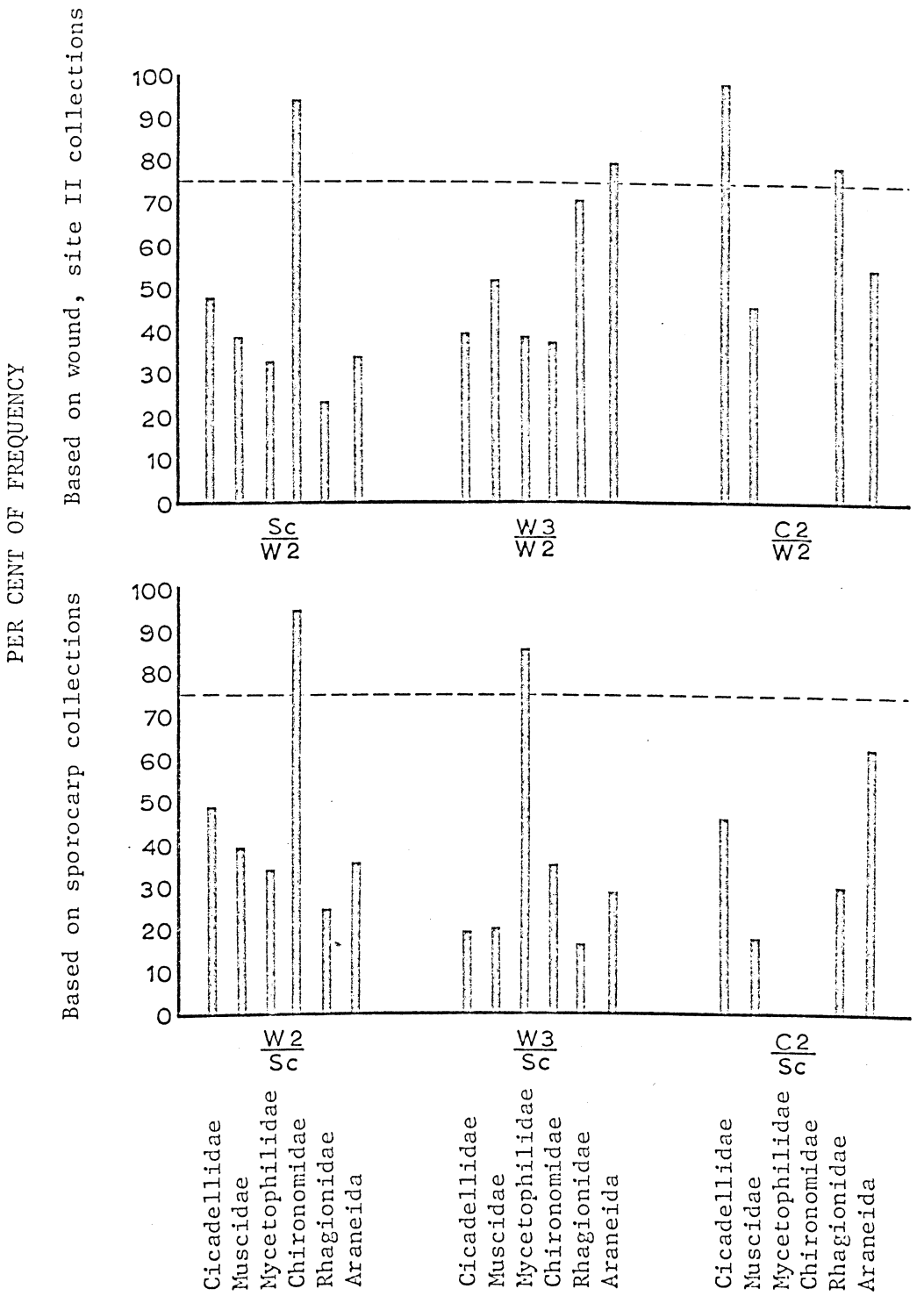


Figure 9. Per cent of times each common group appeared in two sets of traps in 1965. (Sc=Sporocarp traps; W2=Wound traps, site II; W3=Wound traps, site III; C2=Control traps, site II).

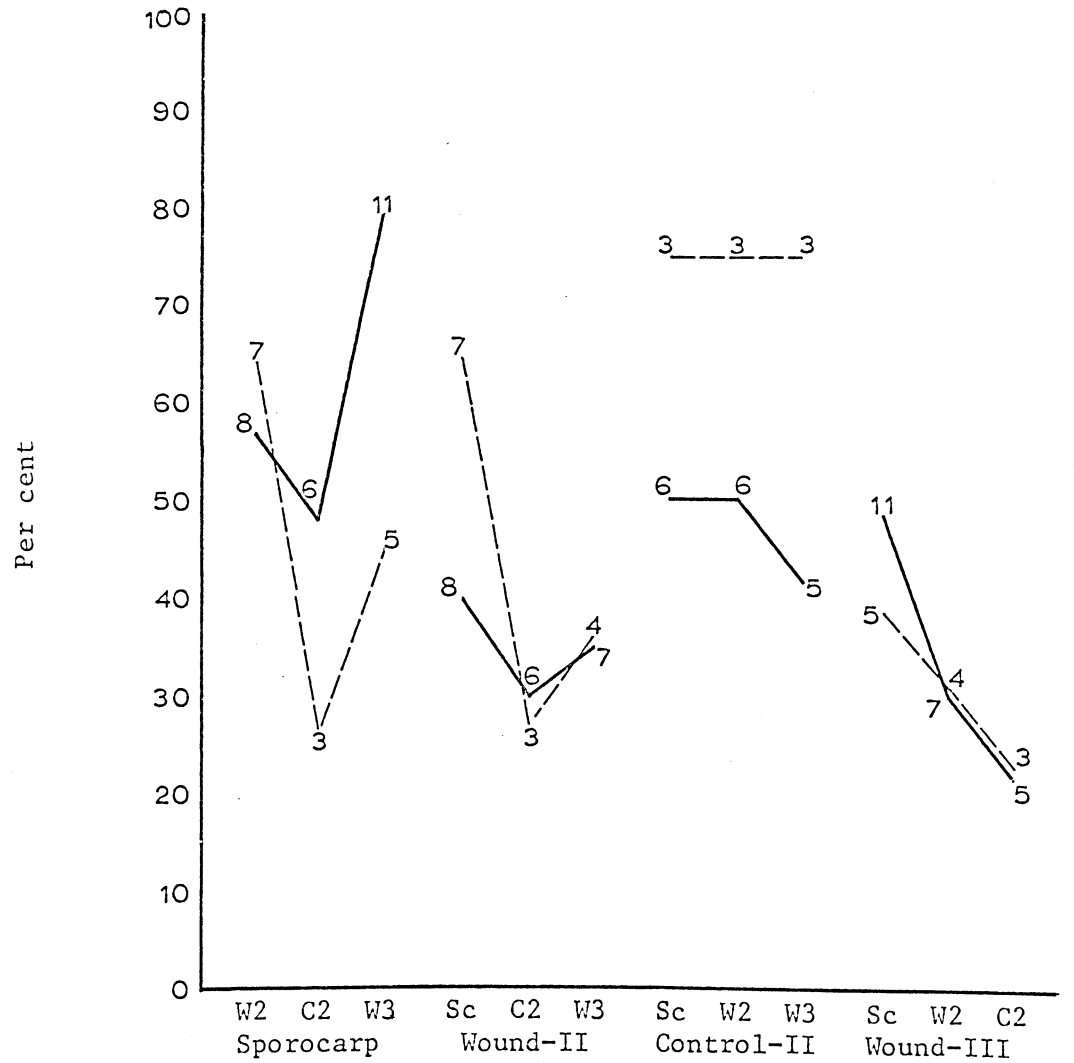


group indicates that it was attracted fairly equally to the two sets of traps being compared. Thus, it is assumed that some individuals of a group, appearing in two sets of traps at a 75 or higher per cent frequency, would be likely to visit traps in both sets during their life span.

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.

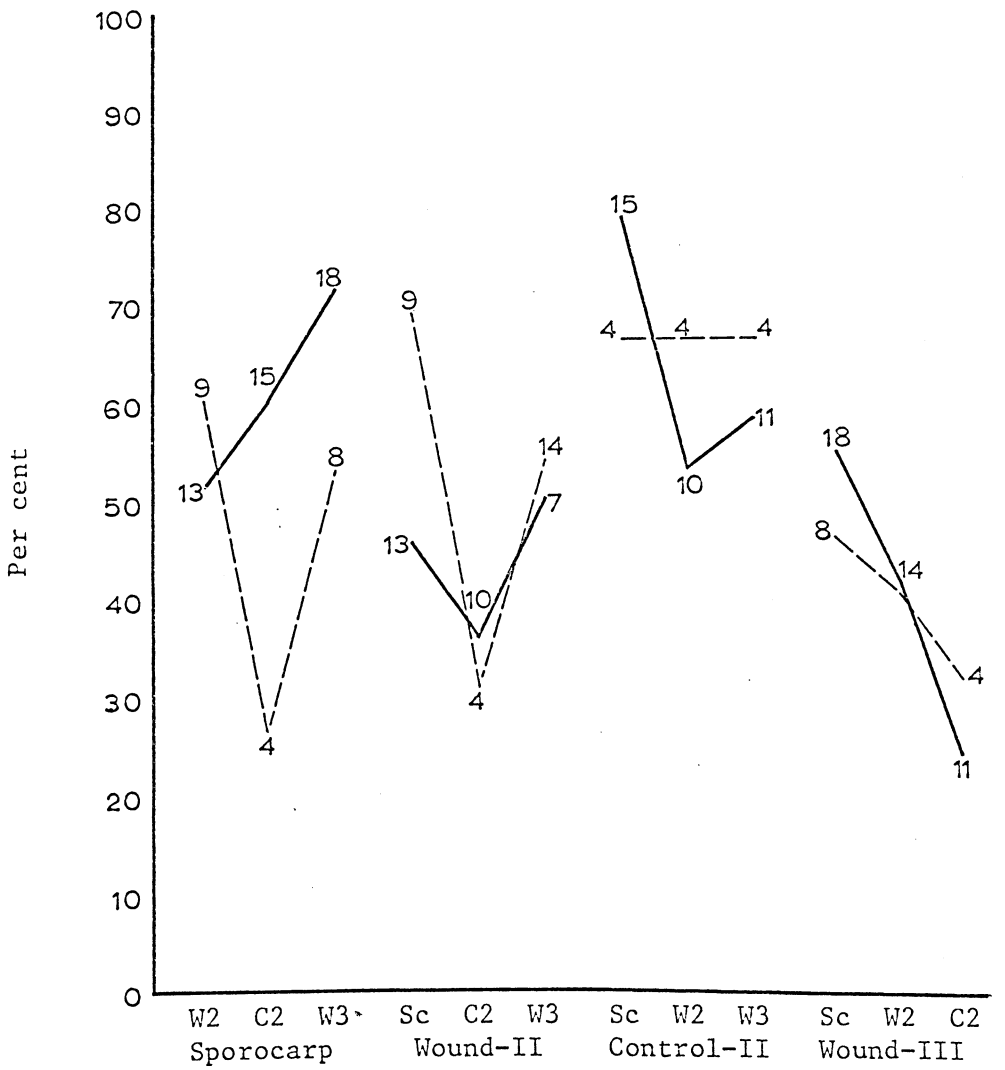
The number of families each of the sets of traps collected in common are compared in Figures 10 and 11. The lines in each section of the graphs show the per cent of families three sets of traps had in common with the fourth, based on the total number of families collected in the latter. The solid lines 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).



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 ragionids 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 compound 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 sporocarp 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 possible 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 sporocarps. Therefore, it can not be concluded that muscids are disseminators, 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. However, 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 normal 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 conclusions 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 dissemination 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 condition 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 mycelium 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 markings 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 between 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 ½ 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 surface 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,

Figure 12. Plastic shell vials, showing method of attachment to the tree.

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 BOLITOTHERUS CORNUTUS AS A POSSIBLE DIRECT DISSEMINATOR OF WOOD ROTTING FUNGI

It is well known that the forked fungus beetle, Bolitotherus cornutus (Panzer) (Tenebrionidae), is associated with many polypores 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 Fomes applanatus. 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 inoculum to suitable inoculation sites.

The investigation of B. cornutus 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 ultraviolet 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 intervales; 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 overwinters 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 protected 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 between 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 exception 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 individual 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 deterioration, 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 entomologists 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 feigning death when disturbed. Other workers have listed B. cornutus as occurring, but not necessarily breeding, on a number of polypores 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 surface 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 Bolitotherus cornutus (X2) on sporocarp of Fomes applanatus. Female is feigning death.

Figure 14. Male Bolitotherus cornutus (X4) on edge of Fomes applanatus 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 individual male or female beetle was often in a mounted position with a different female or male on different occasions. For example, 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 seasons. 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 numbers, 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 relative 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 surviving 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 (unless 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 possibly 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