AN ABSTRACT OF THE THESIS OF

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Title CERTAIN FACTORS AFFECTING COLONIZATION OF PONDEROSA PINE BY IPS CONFUSUS (LECONTE) (COLEOPTERA:SCOLYTIDAE).

Abstract approved [redacted] (Major professor)

The purpose of this study was to study in some detail host factors which influence the population dynamics of bark beetle. The dissertation objectives were to ascertain the applicability of utilizing osmotic pressure values and relative turgidity values of phloem tissue as indicators which reflect upon the physiological profile of the tree; to examine the function of a blue-staining fungus in the bark beetle infested tree syndrome; to investigate dissimilitudes in host physiology and how they correlate to beetle colonization.

The osmotic pressure (o.p.) of the phloem sap in six ponderosa pine were followed from May through September. The initial May values for the three high oleoresin exudation pressure (o.e.p.) trees (greater than 166 p.s.i.) were in excess of 11.2 atmospheres, while the three trees with a zero o.e.p. showed an o.p. of 8.4 atmospheres or less. This distinction between the o.e.p.

categories was not maintained throughout the ensuing four months. The highest o.p. values were observed in July for five of the six trees.

Relative turgidity and phloem thickness measurements did not appear applicable as indicators of the tree's physiological profile. The former measurement was unacceptable due to the large variation in water soluble phloem materials. The latter indicator appeared limited in applicability due to the large variance between trees; however, certain intraspecific trends were established.

Inoculation studies with the blue-staining fungus Ceratocytis ips indicated that the microorganism was aiding colonization and brood development in no other way than through reducing moisture movement and oleoresin exudation in the outer sap wood.

Laboratory studies revealed that Ips confusus prefer higher moisture gradients when the ambient humidity of the ventilating stream in the laboratory olfactometers was low, e.g., 0% R.H. Interference in threshold studies of the attractant principle was avoided by conducting all tests in atmospheres saturated with water vapor.

Five carbohydrates, including maltose, fructose, sucrose, glucose and potato starch, were examined as dietary supplements for enhancing the pheromone synthesis. Data were not conclusive but they suggested that glucose was prominent as a dietary supplement. With the

exception of starch, those sugars containing a glucose moiety showed some effect as a dietary supplement. Subsequent studies on nutritional requirements failed to show that fat soluble materials are a requisite for pheromone synthesis.

Response dissimilitudes in field olfactory studies were related to the osmotic values of the expressed phleom sap. Trees with phloem tissue low in non-electrolytes appeared to represent a substrate which was less favorable as a dietary media for pheromone synthesis. This observation was demonstrated experimentally by severing the sieve elements in ponderosa pine for various periods of time. Olfactometers baited with billets distal to a one-month and one-year-old girdle were preferred by responding Ips confusus. These results were paralleled by comparisons of billets from above and below the site of an infection by Cronartium harknessii.

Dissimilitudes in response patterns were also related to the various heights of the stem from which the billets were obtained. Consistent in two separate studies, male Ips confusus forced into billets from the upper stem portion were capable of eliciting a greater response than males in the butt portion.

The apparent level of pheromone synthesis by male Ips confusus could not be related to the thickness of the phloem. No effect on pheromone synthesis was

apparent when beetles were forced to feed in tissue 0.22 inches thinner than their average dorsal-ventral dimension.

Exuding oleoresin from the exposed surfaces of the billets exhibited a marked interference in field olfactory studies. This interference was reduced by washing the exposed xylem with ethyl alcohol and then applying a coat of molten paraffin.

CERTAIN FACTORS AFFECTING COLONIZATION OF PONDEROSA PINE BY *IPS CONFUSUS* (LECONTE) (COLEOPTERA:SCOLYTIDAE)

by

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CERTAIN FACTORS AFFECTING COLONIZATION OF PONDEROSA PINE BY *IPS CONFUSUS* (LECONTE) COLEOPTERA:SCOLYTIDAE)

INTRODUCTION

The total forested areas of the world cover nearly 14,000 million hectares, of which approximately 50 percent are composed of conifers. A recent meeting of the world's leading forest administrators at the FAO headquarters in New York predicted that the need for forestry products would double in the next 15 to 20 years. The increased requirement, as in the present, will be met primarily by the coniferous forests. The 90 some odd species in the genus *Pinus* L. represent the dominant member of the coniferous forest in value, marketability, but they also sustain the greatest losses as a result of insect depredation.

Bark beetles in the family Scolytidae are probably the most severe insect pests of pine. In the past five decades, a single species of Scolytidae has accounted for an annual loss of ponderosa pine (*Pinus ponderosa* Laws) of about 500 million to 3,500 million board feet (88). Reduction of such losses through conventional control methods, e.g., insecticides, has been precluded by the biology of the insects. Nearly without exception, the major portion of the life cycle is completed in the subcortical tissue, sapwood or reproductive tissues of the host, except for a brief period in which new trees are

colonized. In addition to the biological difficulties associated with bark beetle control, the economic profile often seriously opposes the application of insecticides. The inaccessibility and market value of many timber species do not generally justify a direct therapeutic approach.

Not only has the practicability of forest pesticides been questioned but recent popularization of their potential hazards (18) have resulted in public pressure against their implementation in forest pest outbreaks. In the past, the most successful control measures have been oriented toward preventive rather than direct therapy. The basic principles germane to this approach is the identification and removal of potential breeding material prior to the beetle attack (38, 63 and 113). This technique has limited applicability and apparently is restricted to a single bark beetle species. Through the construction of life tables, attempts have been made to prognosticate future outbreaks, thereby concentrating control efforts in specified areas. In that a depressed physiological condition of the host is vital to a successful bark beetle outbreak, life tables have failed to correlate the numbers of last instar larvae of one population and the numbers of subsequently attacked trees by the succeeding population (109).

The consequence of the above deficiencies has been

the apparent impetus for forest entomological research being oriented toward the investigation of the complex inter-actions in the forest ecosystem. Lucid studies of factors responsible for releasing bark beetle populations are an absolute prerequisite to the development of competent control techniques. Host selection and succeeding colonization, two extremely important phases in the increase of bark beetle populations, have been only poorly investigated up until the last 15 years. Recent studies (1, 110, 129, 130, 131, 133, 134, 135, 140, 141, 142, 143) concerned with beetle dispersion and subsequent concentration on new host material have suggested a new avenue of research which may culminate in an effective control agent.

The unequivocal presence of a pheromone, as defined by Karlson and Butenandt (62, p.39) in Ips pini (Say) (1), Ips confusus LeC. (129), Dendroctonus pseudotsugae (Hopk.) (110), Dendroctonus frontalis Zim. (134) and others, implies that synthetic compounds chemically related to the species-specific pheromone may be effective in concentrating large numbers of bark beetles in a comparatively small area. Hygienic therapy directed towards an endemic population of bark beetles has a theoretically sounder chance of curbing a potential outbreak than control efforts initiated after the extensive phase of the outbreak has been reached. However, due to the apparent

Poisson distribution of a latent population, control attempts directed toward this phase are unrealistic in terms of economical and biological considerations. Concentrating the latent population during the initial dispersal flight, through olfactory stimuli elicited by synthetic pheromones, would simplify and assure a degree of control heretofore unobtainable.

The designs of this dissertation are intended as a furtherance to the existing knowledge concerning the factors which influence the population dynamics of bark beetles. These designs may be summarized as follows: (1) to ascertain the applicability of utilizing relative turgidity values and osmotic pressure values of phloem tissue as indicators which reflect upon the physiological profile of the host; (2) to examine the function of a blue-staining fungus in the bark beetle infested tree syndrome; (3) to investigate dissimilitudes in host physiology and how they correlate to Ips confusus colonization.

LIFE HISTORY OF IPS CONFUSUS

The genus Ips De Geer is one of four members in the tribe Ipini. In North America, 32 species of Ips are recognized. Hopping (56) has divided the genus into ten groups based on the characteristics of the elytral declivity and the sutures of the antennal club. Group Nine, to which Ips confusus has been assigned, is characterized by five spines on each lateral margin of the declivity and the sutures of the antennal club strongly and acutely angled at the middle. All species of this group breed in Pinus species and are restricted to the more arid regions of North America.

Within its range Ips confusus is known to hit all the indigenous species of pine. The principal hosts include Pinus ponderosa, Pinus coulteri D. Don, Pinus radiata D. Don, Pinus lambertiana Dougl., Pinus monticola D. Don, Pinus attenuata Lemm., Pinus balfouriana Murr., Pinus jeffreyi Grev. and Balf., Pinus monophylla Torr. and Frem., and Pinus sabiniana Dougl. In addition, Wood (142) includes Pinus muricata D. Don, and Pinus murrayana Greville and Balfour in the list of preferred pines. The most northern range of Ips confusus appears to be the upper drainage of the Rogue River valley. The distribution extends southward through northern California along the west side of the Sierra Nevada to the Kern River in southern California. Its distribution in the coast

range is limited to the areas south of the Santa Cruz mountains.

The number of yearly generations depends upon elevation and latitude. Five generations may be observed in the southern coast range whereas two are characteristic of the populations in the higher ranges of the north. The winter is passed as either mature larvae pupae or adults. Quiescence is initiated as the result of a cold stupor; consequently, the advent of favorable conditions is sufficient for the resumption of physio- and morphogenesis. A threshold of 18°C., which has also been reported in other bark beetles (111), is required for emergence which may take place as early as March 15.

The attacks, initiated by the emerging spring adults, are typical of the polyogamus genus. The male, responsible for the host selection, usually attacks slash and shaded portions of standing trees. Upon completion of the nuptial chamber, five to eight hours after the initial bark penetration, the male is joined by two to five females. Egg galleries, radiating from the nuptial chamber, often resemble an inverted tuning fork, particularly when three females have constructed galleries.

Eggs are generally laid on both sides of the gallery and require a five to 14 day incubation period. After eclosin, larval maturation occurs on an average of three weeks provided the temperatures are in an excess of 18°C.;

the pupal stage may last up to ten days. Approximately 50 percent of the parent adults, responsible for the first spring attack, re-emerge and initiate new attack. The repetition of this behavorial trait has not been documented but personal observations suggest that at least a third re-emergence and attack may take place.

The attack patterns during the summer are related to the periodic nature of the parent adult and brood emergence. The density of attacks may range from two to 24 per square foot but mass attacks elicited by a feeding stimuli have resulted in densities in excess of 400 adults per square foot (124).

The initial ~~mass~~ attack is carried out by the "pioneer" beetles. These beetles select new host material and subsequently produce a species-specific pheromone. Vité and coworkers (48, 129, 130, 135, 142, 143 and 144) have demonstrated that the apparent biological function of this volatile material is to concentrate the field population on the newly selected tree. This olfactory behavior, as observed for numerous other insects, must be interrupted as a survival mechanism which assures a dispersed population of flying beetles to concentrate on suitable host material.

MATERIALS AND METHODS

Location of Study

The experimentation, unless otherwise noted, was conducted at the Boyce Thompson Institute Forest Research Laboratory and Experimental Forest. The experimental forest, a rectangular, 740 acre block of land, is bordered on the north by the city limits of Grass Valley, California. The forest has an average elevation of approximately 2400 feet with a range of 2500 feet in the northern portion to 2200 feet in the southern portion. The forest cover includes approximately 500 acres of second growth ponderosa pine which ranges in age from 65 to 105 years. In addition to these dominant and codominant members of the stand, the forest contains nearly 100 acres of young jeffrey pine (Pinus jeffreyi Grev. and Balf.), sugar pine (Pinus labertiana Dougl.), and ponderosa pine. Other coniferous species, although at a comparatively low frequency, include Incense cedar (Libocedrus decurrens Torr.), Douglas-fir (Pseudotsugae menziesii (Mirb.) Franco), and white fir (Abies concolor (Gord. and Glend.) Hoppes). The understory is composed of black oak (Quercus kellogii Newberg), Pacific madrone (Arbutus menziesii Pursh), and manzanita (Arctostaphylus manzanita Parry).

Site of Olfactory Studies

All out-door olfactory studies were conducted approximately 50 yards west of the Boyce Thompson Institute Research Laboratory. A concrete slab, constructed with 12 sides and a radius of nearly seven meters (Figure 1) was used as the platform for 12 field olfactometers (Figure 4). Each olfactometer was stationed five meters from the center of the concrete platform. This distance was found (49) to be the optimum radius for recapture of Ips confusus released in the center of the circle. In addition, when the circle of olfactometers was divided into three or four sectors, depending on whether the study was concerned with two or four treatments, olfactometer placement was performed in such a fashion that all treatments were represented in each sector. Moreover, treatments were arranged in each sector so that similar treatments were not in immediate contact. This experimental design proved useful because the wind direction had a marked affect on which sector was most efficient in capturing the field population.

A noticeable difference could be observed in the amount of solar radiation each sector received. To minimize this factor, a 40 x 60 foot net of Saran shade cloth[1] was suspended over the platform at a height of

[1]Saran shade cloth can be purchased from Alapha Hardware, Main Street, Grass Valley, California.

Figure 1. Site of field olfactory studies. Olfactometers were positioned on a concrete slab with a radius of nearly 7 meters and shaded with Saran shade cloth suspended approximately 30 feet above the platform

approximately 30 feet (Figure 1). Thish height did not appear to interfere with the normal flight of the field population. The net is woven so that it imposes a 48 percent reduction on unobstructed sunlight. This net appeared to be instrumental in reducing the sector factor; however, the unidirectional wind did not permit its complete elimination. As a result of this sector factor, full efficiency could not be obtained from all 12 olfactometers.

Experimental Organism

The basis of selection of Ips confusus as the experimental insect, which was used throughout these studies, reflects upon two general points. The first consideration deals with the present status of knowledge pertaining to the life habits of this bark beetle. Data are available (48, 124, 129, 140, 141, 142, 143 and 144) which offer a working knowledge of this insect's life cycle, flight, host selection, and colonization habits.

In addition to this knowledge, it is essential to a study of this nature to have abundant test animals throughout the duration of the investigation. Ips confusus usually has from four to five generations per year in this region; therefore, comparatively high population levels are found in the experimental forest for nearly six months of each year.

The bark beetles used throughout these studies were collected from two sources. The beetles trapped in baited olfactometers were the principal source; however, beetles were collected also from cages which contained infested billets. Data presented in these studies implicating the response patterns of one sex are based on beetles collected from both sources. When no distinction is made as to sex, all data are based on the response patterns of the field population.

Ips confusus collected from the baited olfactometers were preferred for all tests, but inclement weather, as well as periods of low flights, precluded using this type as the sole source. Trapped beetles were augmented with emerged beetles. Small, infested billets from ponderosa pine, usually ranging in size from 15 inches to 24 inches in length and seldom exceeding 12 inches in diameter, were placed in specially designed cages inside of a thermostatically controlled insectary. The insectary was large enough to accommodate four cages measuring 6.5 feet wide, 6.4 feet long, and 6.3 feet high. A strip of tin 14 inches wide was placed at the base of the cage wall which faced the windowed side of the insectary. This smooth surface permitted rapid removal of the emerged beetles.

Collection of emerged beetles was accomplished with a vacuum-cleaner-powered aspirator. The construction of

this apparatus is described in detail by Peterson (103) and requires no further explanation. However, in reference to bark beetles, the use of this apparatus imparts one serious limitation. The beetles are drawn into the apparatus and finally deposited in a pint mason jar which is attached to one end. As a result of this method, beetles are placed in intimate contact with each other. Through continuous mandibular movement, bark beetles, when confined in close association, tend to amputate their appendages. Therefore, a large percentage of the beetles collected are not suitable for olfactory test. Gara (48) demonstrated that removing one antennae reduces the beetle's ability to respond by approximately 50 percent. The amputation of tarsal segments markedly affects Ips confusus' ability to respond to attractants when placed in the laboratory olfactometer.

Olfactory Equipment

Laboratory olfactometer. Past studies (1, 48, 109, 110, 130 and 135) have demonstrated the importance of a volatile material which succors bark beetles in locating trees infested with their own species. This attractive material which guides flight, also appears to have a strong arresting[1] effect in detaining beetles on the tree

[1]The term arresting is not used in the strict sense as defined by Dethier et. al. (35). Instead, its usage is enlarged to include behavior patterns which are characteristic for some bark beetles that are no longer in the flight phase of host selection.

in active search for the entrances to the nuptial chambers or new breeding places. Observations have suggested that searching beetles frequently interrupt their linear progression when in close proximity to newly excavated galleries. Subsequently, klino-taxis movements are observed and many beetles enter the holes.

A laboratory olfactometer was designed which would aid in understanding the response patterns of walking beetles. In addition, it was felt that such an apparatus would be valuable in sustaining principles of response behavior which have been demonstrated in field olfactometers (110, 130, 135 and 144). Conventionally designed "Y" type laboratory olfactometers were not suitable for this purpose. Prevalence of individual differences among bark beetles and frequent distraction from chemotactic response augmented by other stimuli, made such techniques either too complicated or tedious when bioassaying small amounts of volatile material. Wood and Bushing (143) have designed a laboratory olfactometer for studying individual differences but it appeared to be less suitable for testing response behavior of large numbers of beetles.

Figures 2 and 3 show the laboratory olfactometers connected in tandem and the essential internal features. The test arena consists of a masonite peg-board 15 inches square with 3/16 inch holes on one inch centers. Two

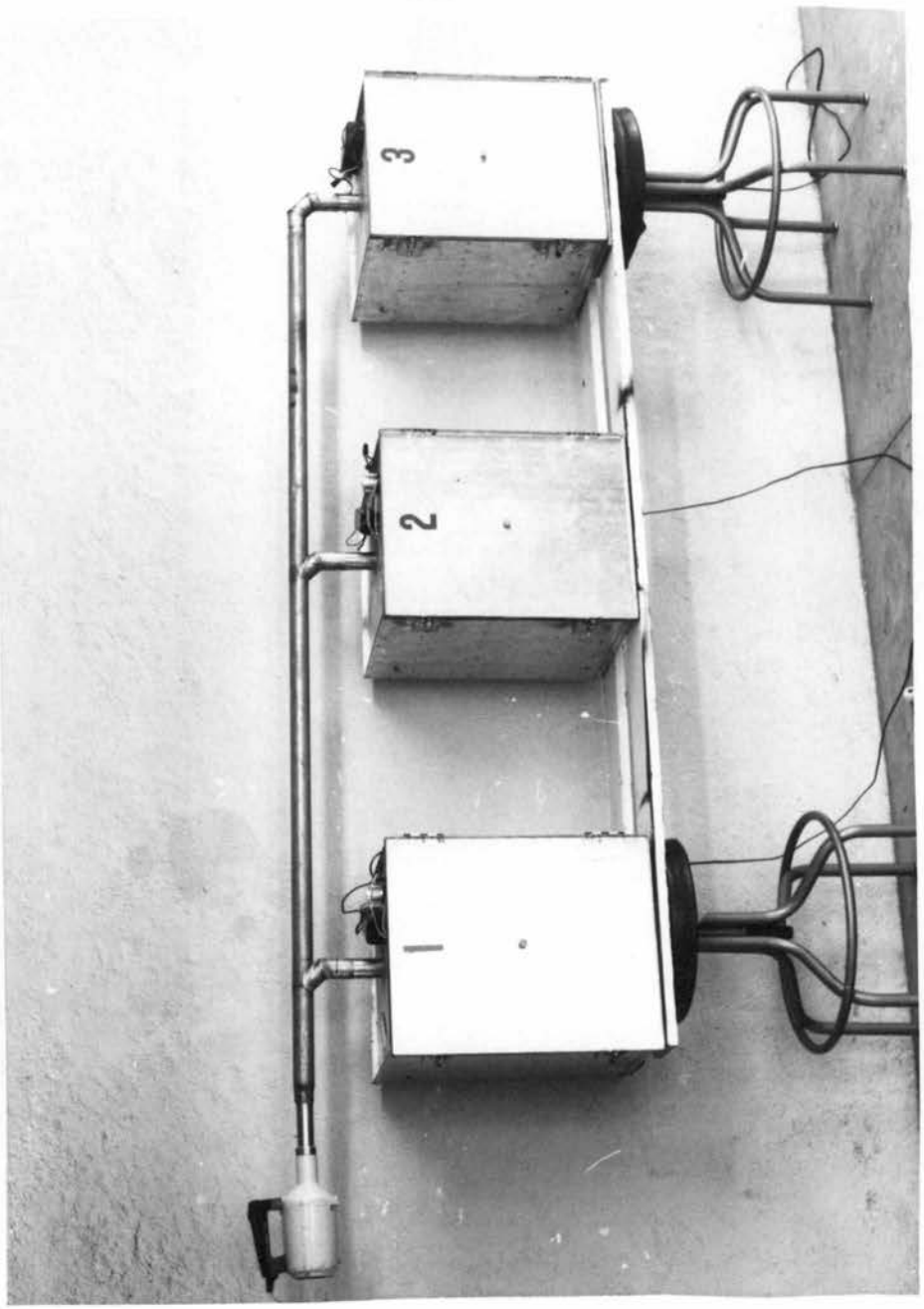

Figure 2. Laboratory olfactometers connected in tandem by a common exhaust vent

Figure 3. Internal features of laboratory olfactometer showing fluorescent ballast and lamp, arena with 12-inch square plastic barrier, small two-inch jars affixed to underside of arena and foam rubber tray used in modifying chamber's air humidity

pieces of peg-board separated by a similar size sheet of window screen were fitted together by wood screws in such a manner that holes in each board coincided. The wire screen prevented beetles from entering holes which were for ventilation and not included in the tests. A double layer of cheesecloth was tightly stretched over the entire arena surface. This material aided the beetles in gaining purchase on the arena surface, prevented accidental entry into holes, but yet did not restrict entry whenever desired. The arena was encircled by a plastic barrier approximately 12 inches square and three inches high confining the beetles to the test area.

Substances to be tested were placed in small jars and attached to the underside of the arena by means of screw caps. Holes were bored through the caps and screen wire; thus, beetles could gain access to the jars after penetrating the cheesecloth barrier. The arrangement of test material used throughout this study was in the form of a circle. This design afforded equal opportunity for the beetles released in the center of the arena to encounter any one of the treatments regardless of the direction of dispersal. The attractive boring dust was tested by placing a small screen reservoir in the mouth of the jars. Thus, contamination of responding beetles and the attractive substances were precluded, permitting the insects to be collected for

subsequent test. Prior to initiating each experiment, the arena and jars were first treated several hours in an oven, set at 75°C., and then a new piece of cheesecloth was stretched over its surface.

Factors such as light, temperature, ventilation and humidity are known to bias response patterns. To reduce the variability of these environmental factors, the arena was enclosed in a 15-1/4 inch x 15-1/4 inch x 20-1/2 inch box constructed from galvanized iron. The front was fitted with a hinged door, facilitating rapid placement and removal of the arena. A circular, 40 watt, cool-white fluorescent lamp, 13 inches in diameter, was attached to the inner top of the box to stimulate locomotion of the test insects. Heat generated by the light was held to a minimum by locating the lamp-ballast outside the box and exhausting air from the box. The inner surface of the box was sprayed with a flat, black paint to avoid reflection. An adequate exchange of air was obtained by connecting the intake of a small vacuum cleaner to a screen-covered hole in the top of the olfactometer box. Air was drawn in from four small holes near the base of each of the three walls. Humidity within the olfactometer chamber was regulated by placing a tightly fitting perforated tray immediately above the intake holes. Material of various types, i.e., anhydrous calcium chloride and foam rubber saturated with water,

were placed in this tray to modify the ambient humidity to the desired level.

Field olfactometers. The olfactometers used throughout this study are essentially the same as the stationary type described by Vité et. al. (135). The use of such a trapping device, as an aid in studying flight habits and response patterns under field conditions, has met with a good deal of success (129, 130 and 134). Gara (48, p.35-36) outlined the essential prerequisites for a successful field olfactometer:

> .... it was important that equipment per se would not be overly repulsive or attractive to the beetles. Only the volatile pheromone could be attractive to the beetles. Second, bait-traps provided with attractants would have to collect responding beetles in an efficient and standardized manner. Third, all beetles would be easily recovered without undue effort or loss of time.

The olfactometer illustrated in Figure 4 appears to fulfill the requirements set forth by Gara. As a result of this enumerated figure, only the salient points of the apparatus will be considered.

The premise for the basic features of this type of field olfactometer stems from the fact that Ips confusus will respond in direct opposition to an air stream carrying its pheromone. The apparatus is composed of three basic parts, each of which is vital in the proper function of the apparatus.

As apparent from the illustration, the large bottom

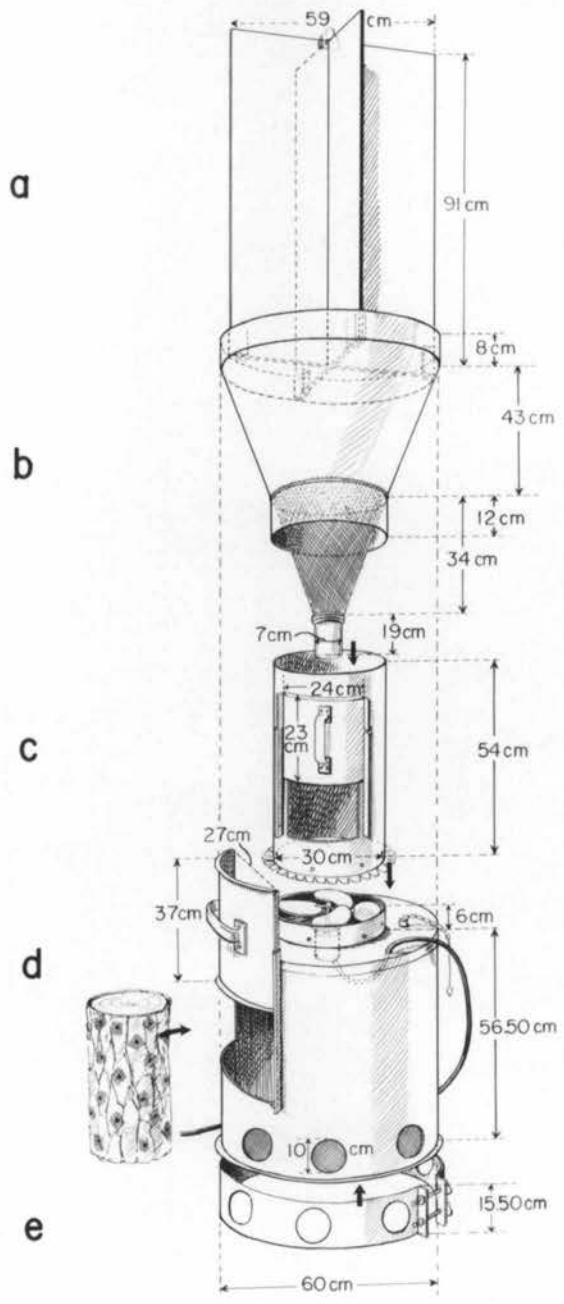

Figure 4. Field olfactometers used throughout this study; (a) peg-board vanes, (b) funnel with screen and collecting jar, (c) pipe section with sliding door, (d) receptacle for infested billets, (e) air stream regulator

section serves as the receptacle air stream regulator for the infested bolt and support for a small 1/30 H.P. electric fan. The fan was capable of delivering a maximum supply of air at a rate of 120 meters per minute and a volume of 25 cubic meters. The supply of air and conversely the rate of flow is controlled by a band of sheet-metal which encircles the screened parts in the base of the olfactometer. The complete closure of these screened parts resulted in the rate of flow being reduced to 30 meters per minute.

The attractant laden air is drawn from the bottom portion of the olfactometer and forced through the middle pipe section and out the large funnel on top. The peg-board vanes extending from the funnel are designed to intercept beetles which fly close to the olfactometer apex. Vité and Gara (129) demonstrated that the size and color of the peg-board vanes affected the efficiency of these trapping devices. During these studies, no effort was made to determine the most optimum vanes; instead all olfactometer vanes were standardized to commercial colored peg-board with a size as shown in Figure 4.

The rate of air delivery to the funnel markedly influences the efficiency of the olfactometer. A relationship exists between flow rate, body weight and flight capacity. Optimum speeds varied with the weight of the insect and its ability. For example, the largest

numbers of Dendroctonus valens Lec. were captured with a flow rate from 90 - 120 meters per minute. The highest frequency of capture occurred with Ips confusus at a flow rate from 60 - 90 meters per second (129). As a result of the above consideration the rate of air flow in all field olfactometers was regulated to a flow from 75 - 85 meters per minute.

Production of the attractant principle. The effectiveness of the field olfactometers depends on forcing attractant laden air through the funnel at the apex of the apparatus. The admixture of the volatile attractant and air occurs in the throat of the olfactometer. The volatile attractant was obtained from the frass of male Ips confusus which had been forced into small ponderosa pine logs.

The procedure followed throughout these studies in forcing male beetles to attack a log was the following. The bark is smoothed to a nearly continuous surface with a 12 inch draw-knife. The bark is then penetrated to the xylem with a blunted, ten-penny nail. Beetles are placed head first in the hole and covered with 18 mesh wire screen. The screen is held in place with staples from a desk stapler. The small logs varied in diameter and length, but rarely exceeded ten inches and 18 inches respectively.

During the earlier phases of this study little if

any attempt was made to standardize the method of selecting and treating logs prior to beetle introduction. However, during the latter phases of this study, trees which were to be used as host material were selected only if the phloem exceeded sixty-thousandths of an inch. In addition, the tree must have had an oleoresin exudation pressure in excess of 90 pounds per square inch. All logs were cut from the lower one-third of the tree stem. No logs were taken from the crown.

As the role of oleoresin became more apparent in our olfactory studies, log sections were not cut until three days after the tree was felled. Ips confusus is definitely sensitive to oleoresin and the presence of this material interferes with the capturing efficiency of the field olfactometers. The interference of oleoresin was reduced by cutting six inch slabs from the face of the logs. The slabs were approximately three inches at their thickest point. The exposed xylem was washed with acetone or ethyl alcohol prior to introducing the male beetle. This procedure was effective in reducing the interference; however, the slabs dried much faster than an intact log. Consequently, the slabs were not suitable for studies which were to exceed a five day duration.

The forced males were effectively producing the attractant within a few hours of introduction. There

appears to be a coincidence between the production of the attractant principle and the construction of the nuptial chamber (130). At least 24 hours were permitted to elapse between tests and the time the beetles were introduced into the slabs or logs. Small logs infested with males in the manner previously described can be expected to be attractive for a period of nearly 23 days; a peak based on numbers of beetles captured occurs between the third and thirteenth day.

The sex of the beetles was verified by the presence of the pars stridens (stridulating organ) in the female beetles (139). Lyon (80) reported a dimorphism on the frons of the males but this technique was less reliable and a good deal slower. Using the stridulating organ, an experienced technician can sex approximately 800 beetles an hour.

The attractive principle tested in the laboratory olfactometers was obtained by forcing males into the host material. The basic variation from field studies was the physical and chemical condition of the host material. Various studies deviated from the standard procedure, but for the most part, host material was treated in the manner outlined by Bedard (4).

Living phloem, which has been recently removed from a tree, is cut into small pieces with a table paper cutter. The next step involves the masticating of the

phloem tissue, with excess distilled water, in a two-speed food blender. When the tissue was reduced to a pulp all excess water was drained off. The pulp was then rinsed with distilled water for approximately five minutes in a 2000 ml. battery jar with continuous agitation. This treatment was repeated three times. The pulp was drained and a quantity placed in the small jars attached to the underside of the laboratory olfactometer arena (Figure 3). The phloem homogenate was pressed with paper towels until, with fairly intense thumb pressure, moisture could no longer be removed from the pulped phloem. Ten holes were pressed into the pulp, at which time male Ips confusus were introduced. No restraining barrier was used to keep the beetles in place. The jars were then incubated at room temperature (24 ± 4°C.) for at least 24 hours.

Osmotic Pressure Studies

A review of the more relevant literature dealing with osmotic pressure studies of living coniferous tissue is given under the result section on physiological indicators. A brief re-examination of the major dictums set forth by these publications appear worth while. Osmotic pressure emerges as one of the more important factors that reflect upon the economy of a tree's moisture supply. Invasion by various organisms is retarded, if not precluded, by oleoresin exudation

pressure. The manifestation of this resistance is dependent upon turgid epithelial tissue lining the resin ducts, which in turn are dependent upon an adequate supply of photosynthates. These dictums prompted a study, design of which was to develop a technique for accurately measuring the osmotic pressure of phloem sap in ponderosa pine.

Explanation of term. The term "osmotic pressure" was originally used to designate a maximum pressure which could develop if the solution was placed in an osmometer. Accurate operation of the osmometer, an apparatus for measuring the magnitude of the osmotic pressure, was possible only under certain ideal conditions. These conditions require a membrane that is permeable only to the solvent, that the osmometer be bathed in the pure solvent and that pressure equilibrium be obtained without appreciable dilution of the solution. Temperature is also a factor because the osmotic pressure of a solution is partially a function of the ambient temperature.

Osmotic pressure values are indices of a potential pressure rather than an existing one and are obtained only under seldom realized conditions. Therefore, the expression may have more biological meaning if it is considered as an index of an osmotically active solution. In actuality, the term characterizes the solution in two ways. The first specification would be the veritable

pressure that the solution could realize if placed in an osmometer under the above specified condition. The osmotic pressure value would also give an index of the diffusion-pressure-deficit; the second specification of the solution. This latter index is a very important utility in understanding the water relationships of a plant.

The simultaneous movement of water within a living system, i.e., two intimately associated cells, is unidirectional and can be predicted. The movement of water under such a system is governed by the cells' diffusion pressure deficit-osmotic pressure-turgor pressure interrelationship. The simple relationship of diffusion-pressure-deficit and osmotic pressure can be given as follows: D.P.D. = O.P. This equation assumes that the volume of the solution cannot appreciably increase; therefore, a cell at dynamic equilibrium with a solvent would have a turgor pressure equal to the osmotic pressure. The osmotic pressure of a solution decreases with dilution; the final osmotic pressure will be proportional to the increase in volume. As a result of this condition the final osmotic pressure and turgor pressure are somewhat less than expected. The equation for biological systems should be expressed as follows: D.P.D. = O.P. - T.P. Under these conditions, water passes through a plant along a gradient of increasing

diffusion pressure deficit.

Osmotic pressure measurements. A change in the osmotic pressure of a solution, i.e., concentration or dilution of the solvent, proportionately influences vapor pressure, boiling point, and freezing point depression of the solution. Thus the mensuration of any of these three physical quantities will give an accurate portrayal of the osmotic pressure. The freezing point depression, or cryoscopic method as it is often called, was used throughout this study.

The cryoscopic determination method is based on the fact that a one molal solution of unionized substance has a theoretical freezing point of -1.86°C. and a theoretical osmotic pressure of 22.4 atmospheres. From these theoretical values the osmotic pressure can easily be derived. The equation for this derivation is shown as:

$$O.P.: 22.4 = \Delta : 1.86$$
$$1.86\ O.P. = 22.4\Delta$$
$$O.P. = \frac{22.4}{1.86}\Delta$$
$$O.P. = 12.04\Delta$$

Δ equals the freezing point depression of a solution. (After Meyer and Anderson 1958 p.91).

The calculation of osmotic pressures using 12.04 is accurate within a few tenths of a percent (76). Meyer and Anderson (87, p.91-92) concurred and stated that this equation appears to be approximately correct over

a wide range of concentrations since deviation from the theoretical osmotic pressures of solutions are accompanied by almost strictly proportional deviations from their theoretical freezing point depression. Findlay (41) has given considerable attention to this subject, but a comprehensive discussion of the physical properties responsible for certain substances deviating from the theoretical freezing point are beyond the scope of this work.

Freezing point depression calculations were obtained with the aid of a Leeds and Northrup No. 8690 millivolt potentiometer (Figure 5). The limits of error of this instrument with reference junction are 0.05% of reading $\pm$ 40 microvolts. The slide wire scale is calibrated from 0-11 millivolts; the smallest division is 0.02 millivolts. It should be pointed out that readings were consistently taken at the third decimal place, thereby greatly increasing the error factor. The procedure was not considered prohibitive because all osmotic pressure determinations are taken as a relative index and not an absolute index.

The instrument was standardized according to the following procedure. The osmotic pressure was determined for each one-tenth molar concentration of reagent sucrose ranging from 1 through .5 molar concentration. The osmotic pressure values were computed and compared

Figure 5. Millivolt potentiometer used for osmotic pressure determinations

against known osmotic pressures. For example, a one molar solution of sucrose was calculated to be 46.17 atmospheres where in actuality it is only 32.05 (41) or 34.6 (127) depending on the source of tabulation.

The correction factor for all calculations was acquired by plotting the differences between observed values and tabulated values. Ursprung's (127) values were used for the correction factor. The differences followed a linear relationship but did not fall on a straight line. To accommodate this fact, a linear regression analysis was applied. The data was transposed to fit a straight line the equation of which is $Y = -.89 + .27X$; the Y axis includes the correction factors in atmospheres and the X axis includes the observed osmotic pressure determinations. Harris and Gortner (52) have calculated the osmotic pressure of vegetable saps from the depression of the freezing point. Osmotic pressure figures can be read directly from their table which includes Δ values from 0.001° to 2.999°.

A wiring diagram of the test junction and reference junction is seen in Figure 6. The test thermocouple (Figure 6C) and reference thermocouple (Figure 6D) are constructed from standard gauge iron and constantan wire. The test thermocouple was formed by bending the end of each wire until it formed an angle of approximately 40°. The union of the two wires was facilitated with a small

drop of flux core solder (Figure 6C). In effect, the thermocouple was formed at the most distal part of the two wires. This scheme permitted the determination of very small amounts of phloem sap. Only three to four drops from a medicine dropper are required when 8 mm O.D. tube is used (Figure 6 E&F). An efficient length for this tube is approximately 140 millimeters (mm). A longer tube would require more sap inasmuch as some of the sap adheres to the tube wall. A shorter tube would increase the time required for the material to freeze.

The freezing chamber unit (Figure 6A) consists of a quart, wide-mouth thermos bottle and contains crushed ice, sodium chloride and occasionally small pieces of solid carbon dioxide. The latter reduces the temperature of the freezing chamber to a lower level, thereby decreasing the time required to freeze a sample. Care must be exercised when solid carbon dioxide is used. If the ice slurry in the freezing chamber becomes solid, the thermos bottle is often ruptured.

The reference junction, formed by twisting the two wires together, is immersed in an ice-water bath (Figure 6B). Once again, a quart thermos bottle serves as the container. This unit is theoretically $0^{o}C$, therefore, sodium chloride is excluded. Under these experimental conditions it was impossible to maintain the reference junction at $0^{o}C$ but it did not exceed

that point by more than .5°C.

As apparent from Figure 6G, the wire common to the test junction is attached to the positive E.m.F. terminal of the potentiometer. Conversely, the wire common to the reference junction is attached to negative E.m.F. terminal. The test junction proved quite satisfactory for this type of temperature determinations. The actual temperature change that occurs when a solution freezes is of a small magnitude and of a comparatively short duration. Thermocouples are aptly suited for this type of study. Their main attributes include their slight size and low heat capacity. The latter permits the test junction to come to rapid thermal equilibrium with the system in which temperature is being measured (43).

Sampling procedure. Prior to initiating the major phase of osmotic pressure determinations, a pilot study was conducted to determine a satisfactory method of procuring phloem samples. In addition, the question remained as to the number of samples necessary for an efficient estimation of this particular parameter.

Development of an adequate sampler was based on two considerations. First, the implement must be easy to handle. Second, the implement must be of the type that permits rapid penetration of xylem to a depth of approximately 2.5 mm. Furniss (46), while working on a sampling technique for the Douglas-fir bark beetle,

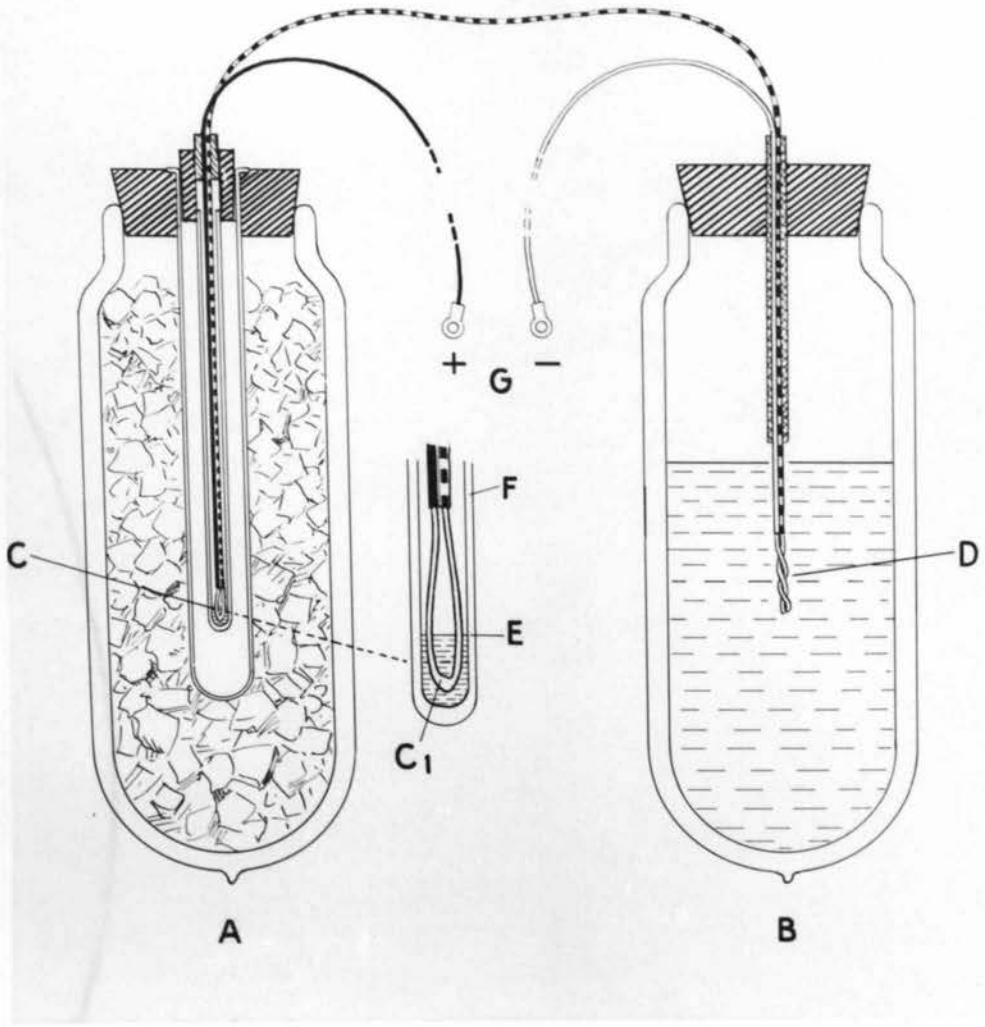

Figure 6. Diagramic explanation of wiring diagram of the test and reference junction used for determining osmotic pressures of expressed phloem cap. (A) freezing chamber, (B) reference chamber, (C and C_1) test junction, (D) reference junction, (E) level of phloem sap, (F) tube used as receptacle for phloem sap, (G) wire leads to E.M.F. terminals

developed a circular punch for obtaining one-tenth square foot bark samples. A punch similar to this type, only of a smaller diameter, was tried; it failed in both specifications. The punch easily penetrated the bark, but was completely ineffective when xylem was encountered.

The ineffectiveness of the punch incited the construction of the samplers seen in Figure 7. Both circular samplers share the same basic design but vary in certain specifications. Each sampler is capable of penetrating the tree to a depth of 40 mm, but the diameter of the smaller sampler is 65 mm and the larger one has a diameter of 85 mm. Both implements have a 12 mm shank faced on one side for efficient operation in a heavy duty, one-half inch, reversible electric drill. The distal end of the shank is modified to accommodate a 7.5 mm, high speed, steel bit. The bit serves as a guide during the initial phase of thebark penetration. The power supply was furnished by a portable, gasoline operated generator capable of delivering a 115 volt, alternating current rated at 15.2 amperes.

After the tree has been penetrated to the depth of the circular sampler, a one-half inch chisel is used to remove the wooden disc. Care should be used to avoid separating the xylem from the inner bark. Phloem tissue which is exposed to the air rapidly loses moisture and results in a higher osmotic pressure determination.

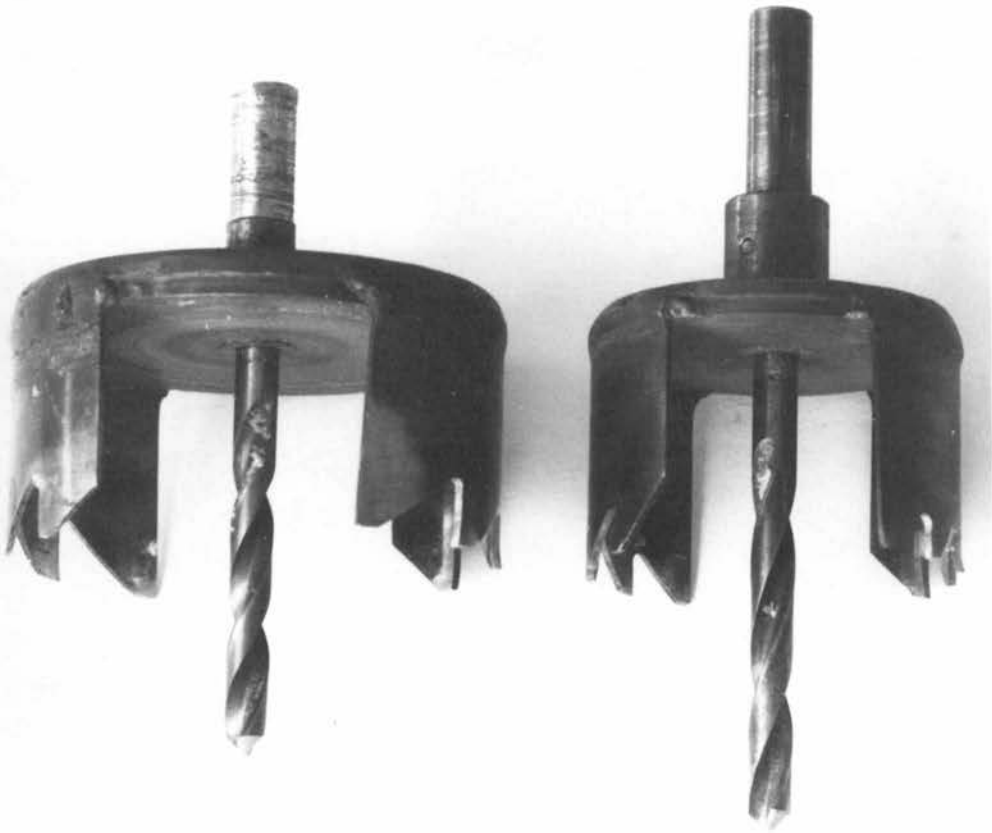

Figure 7. Circular samplers of 85 mm. and 65 mm. used for procuring phloem tissues in osmotic pressure studies

Each wooden disc, once removed from the tree, was wrapped in aluminum foil, thereby reducing excessive moisture loss. The wooden discs were placed in plastic bags and stored in a freezer set at -10°F until the samples could be processed.

The wooden discs were allowed to thaw at room temperature one to two hours prior to separating the xylem and phloem. Phloem tissue, as free as possible from xylem and bark, was pressed in a Carver press at a pressure of 20,000 pounds per square inch. This high pressure was necessary when the phloem was very thin. Thin phloem is generally characterized by a low sap content; based on a comparison of equal units of thin and thick phloem. The press was tilted forward at a slight angle. This technique resulted in sap accumulating toward the front of the metal dish. All units of the press coming in contact with the sap were washed in warm soapy water after each sample was processed. Both new medicine droppers and sample tubes were used for each phloem sample, thereby precluding equipment contamination.

The question still remained as to the number of samples necessary for an efficient estimation of the osmotic pressure parameter. Three trees were selected at random from a population of ten trees whose oleoresin exudation pressure exceeded 100 pounds per square inch.

The method for oleoresin exudation pressure determination is reported in another study (128). A total in excess of 39 samples was taken from each tree in a band, center of which was approximately at breast height.

The osmotic pressure was determined in the manner previously described. Average osmotic pressures in atmospheres were 16.5, 14.1 and 16.7 with a sample size of 48, 43 and 40 respectively. Sample variances (s^2) determined according to Li's (77, p.62) procedure were 2.35, 4.38 and 8.36 respectively. As a result of these large variances plus the average number of samples which could be processed in one day (approximately 35), a sample size of 7 was chosen.

Phloem Thickness Measurements

Studies on differences in host physiology suggest that frass produced by male Ips confusus can elicite different response patterns; these disparities can be attributed, at least in part, to dissimilitudes in phloem physiology. During the course of studies on host physiology it became essential to investigate the influence of phloem thickness on Ips confusus' ability to produce the attractant principle.

A "Starrett" paper, gage micrometer catalogue number 223 RL, size 11/32 of an inch (Figure 8) was employed in estimating phloem thickness. The construction

Figure 8. Paper gauge micrometer used for measuring the thickness of phloem tissue

of this instrument incorporates two vital features which are important to this type of study. The measuring surface must be large because living phloem is quite undulated. The broad anvil and spindle of a paper gage micrometer reduces the error in estimating phloem thickness. This type of measuring calipers is equipped with a ratchet stop. Living phloem can be compressed without an undue amount of pressure; however, the ratchet stop allows a comparable amount of pressure to be applied at each reading.

Samples were generally taken at breast height. Phloem was procured with the aid of the 65 mm circular sampler (Figure 7). The phloem was carefully separated from the wood and bark on one-half of the wooden disc. A single reading was then taken from the area with thickest phloem. This particular instrument is accurate up to .001 inch. To assure accuracy, the anvil and spindle surfaces should be cleaned occasionally with an organic solvent.

Oleoresin Exudation Measurements

The use of oleoresin exudation pressure (o.e.p.) (128, 131, 132, 133 and 141) has proven a useful tool in characterizing certain physiological types in ponderosa pine. Vité (128) pioneered the measurement of oleoresin pressure in ponderosa pine and was able to demonstrate

the interrelationship of water balance, o.e.p., and tree resistance. When the water balance of a tree is disturbed a marked change in o.e.p. follows. With some trees this reduction is temporary and only a period of transition prior to establishing a favorable balance, i.e., dropping of needles. However, with other trees this reduction of pressure appears to be chronic. The point was reasoned that trees with a chronically depressed water balance must differ in certain other physiological attributes. As a result of this reasoning marked differences, i.e., o lbs. and 100 lbs., in o.e.p. were used as the initial criterion in tree selection.

The manner in which o.e.p. was determined is essentially the same as described by Vité (128, p.42) except for a few minor modifications. Hydrostatic gauges of Bourdon type, calibrated for pressures of 0-200 pounds per square inch, were used throughout these studies (Figure 9). When it became essential to establish the absolute maximum pressure, under the existing conditions, gauges equipped with an additional maximum pressure indicator were used (Figure 9). This indicator remained at the highest registered pressure. In general, these types of gauges are not accurate at pressures below 20 p.s.i. To accommodate for this error, up to two additional gauges were used when the initial reading was below 20 pounds.

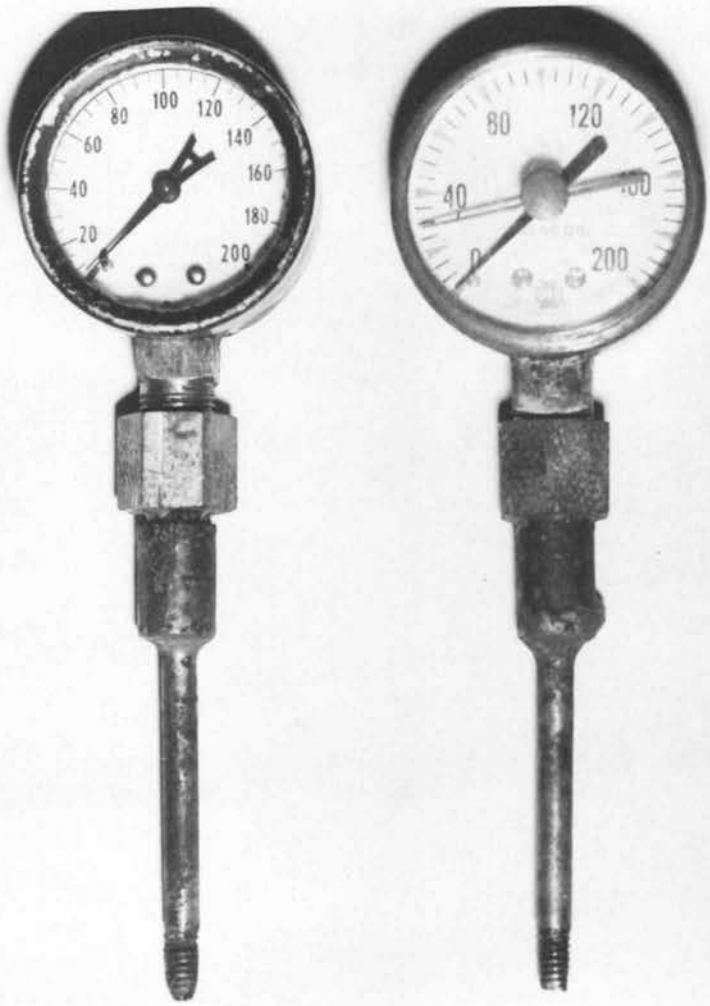

Figure 9. Bourdon type hydrostatic gauges used for oleoresin exudation pressure measurements. Gauge on right is equipped with an additional maximum pressure indicator which remains at the highest pressure

The procedure for affixing the gauge to the tree has been described elsewhere (128); therefore, only salient points and modifications will be considered. The outerbark is chipped away until a depth of approximately one inch remains. A wood brace with a three-eighths inch, slow speed steel bit is then employed to penetrate the xylem to a depth of approximately two inches. The hole, drilled at a slight angle, is filled with glycerol. A two and one-half inch metal nipple of one-eighth inch pipe, connected to the gauge by a reducer and adapter, is screwed into the hole to a depth of nearly one and one-half inches. Both the gauge and nipple have been filled with glycerol, thereby facilitating rapid equilibrium of actual pressure and recorded pressure.

The chemical properties of oleoresin cause crystallization once the fluid has been exposed to atmospheric air, thereby precluding accurate pressure determinations 24 hours after the gauges were installed. For the most part, gauges were affixed to trees at breast height in the afternoon preceding the day on which readings were recorded. The maximum pressure was the basis of selection. Readings consequently were obtained from all gauges not equipped with a maximum pressure indicator immediately prior to sunrise.

Blue-Stain Studies

One phase of this study was concerned with the association of Ips confusus and blue-staining fungi. A survey by Mathre (81) on fungi associated with bark beetles demonstrated the apparent specificity of the fungus-beetle relationship. Mathre, after studying two locations in the northern coast range of California and eight locations in the Sierra Nevada mountains from Mt. Lassen to Sequoia National Park, concluded that the specificity of association between bark beetles and certain members of the genus Ceratocystis appears to be fairly constant. In direct reference to Ips, he adds that the beetles are to be found associated only with Ceratocystis ips. Based on these findings a study was undertaken to consider the role of this organism in colonization of new breeding material by Ips confusus.

The majority of the experimentation was concerned with the effect of blue-stain per se rather than in association with the bark beetle. Ideally, it would be desirable to study both organisms independent of the other's influence, but time and facilities precluded the development of a colony of aseptic bark beetles. Artificial inoculation was attained with an inoculum of either a watery suspension containing blue-stain perithecia or shakes cultures of conidia and mycelium in potato broth. No effort was made to determine the

pathenogenicity of the two inoculums.

All artificial inoculations were achieved by removing the bark and phloem from the whole circumference of the tree in a band at least 40 centimeters wide. These bands were immediately wrapped with cellucotton and moistened with one of the above mentioned inoculums. Excessive moisture loss from the inoculation band was reduced by covering the region with polyethylene sheets.

The selection of host material to be inoculated was based on the maximum o.e.p. Trees were arbitrarily divided into categories depending upon their maximum pressure. The study concerned with the blue-stain-bark beetle-o.e.p. interrelationship required that high pressure trees, i.e., 100 p.s.i. be reduced to 0 p.s.i. Oleoresin exudation pressure was reduced successfully by applying a basal treatment of herbicide containing a mixture of 2,4-D (22.6%) and 2,4-T (10.8%) in kerosene or diesel oil at one pint to two and one-half gallons respectively. Within a few hours after the application of the herbicide, trees were characterized by a sharp rise in o.e.p. The maximum may occasionally exceed 200 p.s.i. Following this rapid rise, there was a constant decrease in pressure marked by a series of wide fluctuations. After a period of two weeks the o.e.p. can no longer be detected by the previously described method.

Statistical Analysis

An essential requirement in olfactory studies of this type is that all test beetles have equal opportunity to encounter any particular treatment. This takes into account beetles that are outside or inside the perimeter of treatments. As a direct result of this requisite, a modified Latin square was exploited as the experimental design. The basic deviation from the Latin square was the spacial arrangement of the treatments. Instead of incorporating treatments in blocks and columns they were systematized in the form of a circle. The sequential placement of treatments was directed by the number of treatments and the requirement that similar treatments do not occur in unison.

Nearly all experimentation was concerned with the effect of testing four treatments simultaneously. Occasionally, however, only two treatments were studied. To accommodate the former number of treatments and still maintain the requisite that bias be eliminated from treatment placement, the circle was constructed with 12 positions. As a consequence, the circle was arbitrarily divided into three sectors, each of which contained all four treatments. When the results were based on two treatments the circle was divided into four sectors; this particular design utilized eight positions. Prior to initiating an explanation on statistical procedures

it is important to point out that individual treatments within each sector were not considered as replications.

Two statistical techniques were used throughout these studies. The "Student's" t-test was employed as a criterion for the significance of two sample means. The comparison of two sample means is considered throughout these tests as unpaired data. Accordingly, the t-test for unpaired data, as outlined by Johnson (6o, p.36), was selected as the comparison test. The second statistical technique utilized was the factorial analysis of variance. All sums ofsquares and mean square values found in analysis of variance tables were obtained from the 1620 IBM computer which is maintained by the Statistic Department at Oregon State University.

Certain limitations and assumptions are inherent to both statistical techniques. These factors reflect upon the accuracy of inductive generalizations; consequently, a declaration on the interpretation of significant probabilities appears necessary.

Simpson et. al. (119, p.183-4) defines the assumptions of the t-test. The first is that the population from which the samples are drawn be normal. The second underlying assumption is that the variance of the two populations are equal. In practice, only the latter assumption is important in the t-test. Simpson et. al. (119, p.184) states:

> ... the t-test, as given in this book, does test only the differences between the means, and it requires a very large difference between the variances of the population to have any effect. It is, of course, conceivable that some populations might have the same mean but such radically different variances as to produce a significant value of t.

They conclude that when the variance of two populations are thought to be equal, the best procedure is to exercise caution in inductive generalizations, especially when the probability is close to the selected significance level. Consideration of these assumptions and limitations has resulted in the acceptance of the null hypothesis when calculated t values are significant at the 5% level. All probability values are computed on the basis of a one-side test.

Although the same limitations apply to the analysis of variance test, the important issue with this technique appears to be the nature of the factors[1] being analyzed. The two types which affect the interpretation of any factorial analysis of variance test are random and fixed factors. The distinction between these factors results in the possibility of three basic analyses of variance models, e.g., random, fixed, and mixed. All olfactory studies conducted with field and laboratory olfactometers

[1]The term 'factor' may be considered synonymous with treatment, but it is expanded in these studies to include such elements as time, days, sectors and replications.

necessitate an analysis of variance test using a mixed model.

Factors were considered random or fixed according to the definitions set forth by Simpson et. al. (119, p.265). They approximate the rules of decision by stating:

> If the observation were to be made several times, would the biology of the problem force the same choice of levels[1] each time, or would any set of levels work? If the choice of levels is a fixed one; if the choice of levels is arbitrary, it is a random factor.

In olfactory studies conducted in the field, factors such as sectors, time and days are considered as random; treatments are considered fixed. All factors in the laboratory studies are considered fixed.

The recognition of a mixed model is essential in selecting the proper denominator for determining F values. The residual mean square value (error) cannot always be used. The proper F ratio can be easily determined with the aid of the expected mean squares. The rule (119, p.297) states that the numerator and denominator of F must differ only by a quantity proportional to the component being tested. Cornfield and Tukey (31) have described the method of computing the average value of any mean square. Tables 1, 1a, 2, and 2a show the computation of average mean square values for factorial

[1]Level: number of varieties in each factor; i.e., time - 1000, 1200, 1400, etc.

analysis of variance with mixed models including one fixed and three random factors, and two fixed and three random factors. The numerals represent factors, the letters represent levels; e.g., 1-a, 2-b, 3-c, 4-d, etc.

Table 1. Average mean squares of a 1 fixed and 3 random factor model assuming fixed-random factor interaction

Source	Average mean square
1 effect(fixed)	$\sigma_e^2 + n\sigma_{1234}^2 + nd\sigma_{123}^2 + nc\sigma_{124}^2 + nb\sigma_{134}^2 + nbd\sigma_{13}^2 + nbc\sigma_{14}^2 + nbcd\sigma_1^2$
2 effect(random)	$\sigma_e^2 + na\sigma_{234}^2 + nad\sigma_{23}^2 + nac\sigma_{24}^2 + nacd\sigma_2^2$
3 effect(random)	$\sigma_e^2 + na\sigma_{234}^2 + nad\sigma_{23}^2 + nab\sigma_{34}^2 + nabd\sigma_3^2$
4 effect(random)	$\sigma_e^2 + na\sigma_{234}^2 + nac\sigma_{24}^2 + nab\sigma_{34}^2 + nabc\sigma_4^2$
1x2 interaction	$\sigma_e^2 + n\sigma_{1234}^2 + nd\sigma_{123}^2 + nc\sigma_{124}^2 + ncd\sigma_{12}^2$
1x3 interaction	$\sigma_e^2 + n\sigma_{1234}^2 + nd\sigma_{123}^2 + nb\sigma_{134}^2 + nbd\sigma_{13}^2$
1x4 interaction	$\sigma_e^2 + n\sigma_{1234}^2 + nc\sigma_{124}^2 + nb\sigma_{134}^2 + nbc\sigma_{14}^2$
2x3 interaction	$\sigma_e^2 + na\sigma_{234}^2 + nad\sigma_{23}^2$
3x4 interaction	$\sigma_e^2 + na\sigma_{234}^2 + nab\sigma_{34}^2$
1x2x3 interaction	$\sigma_e^2 + n\sigma_{1234}^2 + nd\sigma_{123}^2$
1x2x4 interaction	$\sigma_e^2 + n\sigma_{1234}^2 + nc\sigma_{124}^2$
1x3x4 interaction	$\sigma_e^2 + n\sigma_{1234}^2 + nb\sigma_{134}^2$
2x3x4 interaction	$\sigma_e^2 + na\sigma_{234}^2$
1x2x3x4 residual	$\sigma_e^2 + n\sigma_{1234}^2$

Table 1a. Average mean squares of a 1 fixed and 3 random factor model assuming no fixed-random factor interaction

Source	Expected mean square
1 effect (fixed)	$\sigma_e^2 + n\sigma_{1234}^2 + nbcd\sigma_1^2$
2 effect (random)	$\sigma_e^2 + na\sigma_{234}^2 + nacd\sigma_2^2$
3 effect (random)	$\sigma_e^2 + na\sigma_{234}^2 + nabd\sigma_3^2$
4 effect (random)	$\sigma_e^2 + na\sigma_{234}^2 + nabc\sigma_4^2$
2x3 interaction	$\sigma_e^2 + na\sigma_{234}^2 + nad\sigma_{23}^2$
2x4 interaction	$\sigma_e^2 + na\sigma_{234}^2 + nac\sigma_{24}^2$
3x4 interaction	$\sigma_e^2 + na\sigma_{234}^2 + nab\sigma_{34}^2$
2x3x4 interaction	$\sigma_e^2 + na\sigma_{234}^2$
1x2x3 residual	$\sigma_e^2 + n\sigma_{1234}^2$

Table 2. Average mean squares of a 2 fixed and 3 random factor model assuming fixed-random factor interaction

Source	Expected mean square
1 effect (fixed)	$\sigma_e^2 + n\sigma_{12345}^2 + ne\sigma_{1234}^2 + nd\sigma_{1235}^2 + nc\sigma_{1245}^2 + nb\sigma_{1345}^2 + ncd\sigma_{125}^2 + nce\sigma_{124}^2 + nde\sigma_{123}^2 + nbd\sigma_{135}^2 + nbe\sigma_{134}^2 + ndce\sigma_{12}^2 + nbde\sigma_{13}^2 + nbce\sigma_{14}^2 + nbcd\sigma_{15}^2 + nbcde\sigma_1^2$
2 effect (fixed)	$\sigma_e^2 + n\sigma_{1234}^2 + ne\sigma_{1234}^2 + nd\sigma_{1235}^2 + nc\sigma_{1245}^2 + na\sigma_{2345}^2 + ncd\sigma_{125}^2 + nce\sigma_{124}^2 + nde\sigma_{123}^2 + nad\sigma_{235}^2 + nac\sigma_{234}^2 + ndce\sigma_{12}^2 + nade\sigma_{23}^2 + nace\sigma_{24}^2 + nacd\sigma_{25}^2 + nacde\sigma_2^2$
3 effect (random)	$\sigma_e^2 + na\sigma_{2345}^2 + nae\sigma_{234}^2 + nad\sigma_{235}^2 + nab\sigma_{345}^2 + nabe\sigma_{34}^2 + nabd\sigma_{35}^2 + nabde\sigma_3^2$
4 effect (random)	$\sigma_e^2 + na\sigma_{2345}^2 + nae\sigma_{234}^2 + nac\sigma_{245}^2 + nab\sigma_{345}^2 + nabe\sigma_{34}^2 + nabc\sigma_{45}^2 + nabce\sigma_4^2$
5 effect (random)	$\sigma_e^2 + na\sigma_{2345}^2 + nad\sigma_{235}^2 + nac\sigma_{245}^2 + nad\sigma_{345}^2 + nabd\sigma_{35}^2 + nabc\sigma_{45}^2 + nabcd\sigma_5^2$
1x2 interaction	$\sigma_e^2 + n\sigma_{12345}^2 + ne\sigma_{1234}^2 + nd\sigma_{1235}^2 + nc\sigma_{1245}^2 + ncde\sigma_{12}^2$

Table 2. (Continued)

Source	Expected mean square
1x3 interaction	$\sigma_e^2 + n\sigma_{12345}^2 + ne\sigma_{1234}^2 + nd\sigma_{1235}^2 + nb\sigma_{1345}^2 + nbde\sigma_{13}^2$
1x4 interaction	$\sigma_e^2 + n\sigma_{12345}^2 + ne\sigma_{1234}^2 + nc\sigma_{1245}^2 + nb\sigma_{1345}^2 + nbce\sigma_{14}^2$
1x5 interaction	$\sigma_e^2 + n\sigma_{12345}^2 + nd\sigma_{1235}^2 + nc\sigma_{1245}^2 + nb\sigma_{1345}^2 + nbcd\sigma_{15}^2$
2x3 interaction	$\sigma_e^2 + n\sigma_{12345}^2 + ne\sigma_{1234}^2 + nd\sigma_{1235}^2 + na\sigma_{2345}^2 + nade\sigma_{23}^2$
2x4 interaction	$\sigma_e^2 + n\sigma_{12345}^2 + ne\sigma_{1234}^2 + nc\sigma_{1245}^2 + na\sigma_{2345}^2 + nace\sigma_{24}^2$
2x5 interaction	$\sigma_e^2 + n\sigma_{12345}^2 + nd\sigma_{1235}^2 + nc\sigma_{1245}^2 + na\sigma_{2345}^2 + nacd\sigma_{25}^2$
3x4 interaction	$\sigma_e^2 + na\sigma_{2345}^2 + nae\sigma_{234}^2 + nabe\sigma_{34}^2$
3x5 interaction	$\sigma_e^2 + na\sigma_{2345}^2 + nad\sigma_{235}^2 + nabd\sigma_{35}^2$
4x5 interaction	$\sigma_e^2 + na\sigma_{2345}^2 + nac\sigma_{245}^2 + nabc\sigma_{45}^2$
3x4x5 interaction	$\sigma_e^2 + n\sigma_{1234}^2 + na\sigma_{2345}^2 + nab\sigma_{345}^2$
Second order interactions[2]	
1x2x3x4x5 residual[3]	$\sigma_e^2 + n\sigma_{12345}^2$
Third order interactions[2]	

[1] n = number of replications. [2] Remainder of expected mean squares omitted. [3] Used as error term.

Table 2a. Average mean squares of a 2 fixed and 3 random factor model assuming no fixed-random factor interaction

Source	Expected mean square
1 effect (fixed)	$\sigma_e^2 + n\sigma_{12345}^2 + nbcde\sigma_1^2$
2 effect (fixed)	$\sigma_e^2 + n\sigma_{12345}^2 + nacde\sigma_2^2$
3 effect (random)	$\sigma_e^2 + na\sigma_{2345}^2 + nabde\sigma_3^2$
4 effect (random)	$\sigma_e^2 + na\sigma_{2345}^2 + nabce\sigma_4^2$
5 effect (random)	$\sigma_e^2 + na\sigma_{2345}^2 + nabcd\sigma_5^2$
1x2 interaction	$\sigma_e^2 + n\sigma_{12345}^2 + nade\sigma_{12}^2$
3x4 interaction	$\sigma_e^2 + na\sigma_{2345}^2 + nabe\sigma_{34}^2$
3x5 interaction	$\sigma_e^2 + na\sigma_{2345}^2 + nabd\sigma_{35}^2$
4x5 interaction	$\sigma_e^2 + na\sigma_{2345}^2 + nabc\sigma_{45}^2$
3x4x5 interaction	$\sigma_e^2 + na\sigma_{2345}^2$
1x2x3x4x5 residual	$\sigma_e^2 + n\sigma_{12345}^2$

As apparent from Tables 1 and 2, when Simpson's et. al. rule for determining the proper F ration is followed, a denominator is not available for several of the main effects. This fact has resulted in the construction of Tables 1a and 2a in which fixed-random factor interaction are considered to equal zero. By making this assumption, either the highest order

interaction of the random factors or the residual becomes the appropriate denominator for the F ratio.

The fact should be pointed out that in many analyses shown in this work, fixed-random factor interactions are significant and not equal to zero. Simpson et. al. (119, p.296) considers this limitation in their statement:

> There are objections to this procedure, the chief one being that it is somewhat biased. At times, the interaction component which was assumed to be zero will really exist, and this will result in an overestimate of the main effect. This defect in the method is offset by others in the more complex technique of manipulating the various means squares to find a suitable denominator.

As a corollary of the tendency to overestimate main effects, the null hypothesis is accepted in all mixed models when the probability is significant at the 5% level.

RESULTS AND CONCLUSIONS

Physiological Indicators

Osmotic pressure. The measurement of oleoresin exudation pressure (o.e.p.) has proven an effective tool in portraying the water balance of a tree (90 and 128) and prognosticating its susceptibility to bark beetle attack (133 and 141). The principle germane to the utilization of this tool is found in the oleoresin exudation potential of a tree. The o.e.p. is dependent upon turgid resin channel epitheliun which in turn are dependent upon a favorable water balance. In addition to moisture, turgidity is also related to osmotically active materials, e.g., photosynthates. The point was reasoned that trees with low o.e.p. may have an accompanying low o.p. of the expressed phloem sap.

To pursue this line of reasoning, six trees were selected which appeared to be representative of their particular o.e.p. group. Verification of this theory appeared to require that trees with an initial low o.e.p. possess a low o.p. and maintain this correlation throughout the major portion of the season. As a result, the selected trees included three with an o.e.p. in excess of 166 p.s.i. and three with an o.e.p. of zero. The object of this study was to follow the seasonal o.p. pattern in order to determine if monthly observation changed from

May through September.

A few of the salient features of the trees studied are given in Table 3. The method for obtaining samples and the procedure for o.p. determination has been considered in an earlier section. The monthly o.p. value for each tree is based on the mean of seven samples.

Table 3. Characterization of ponderosa pine used in seasonal o.p. study

Tree	o.e.p. (p.s.i.)	Height (feet)	Age (years)	d.b.h. (inches)
A	0 [1/]	84	71	6.0
B	0 [1/]	73	68	7.0
C	0 [1/]	62	82	10.2
D	175	77	79	12.2
E	170	69	64	9.5
F	167	77	74	12.0

[1/] Based on three readings.

The results of this study are shown graphically in Figure 10. The relationship between high and low pressure trees in terms of o.p. is not distinct but a general pattern appears to be present. The two categories of trees in May are characterized by a marked difference in o.e.p.; this disparity also is manifested by initial o.p.

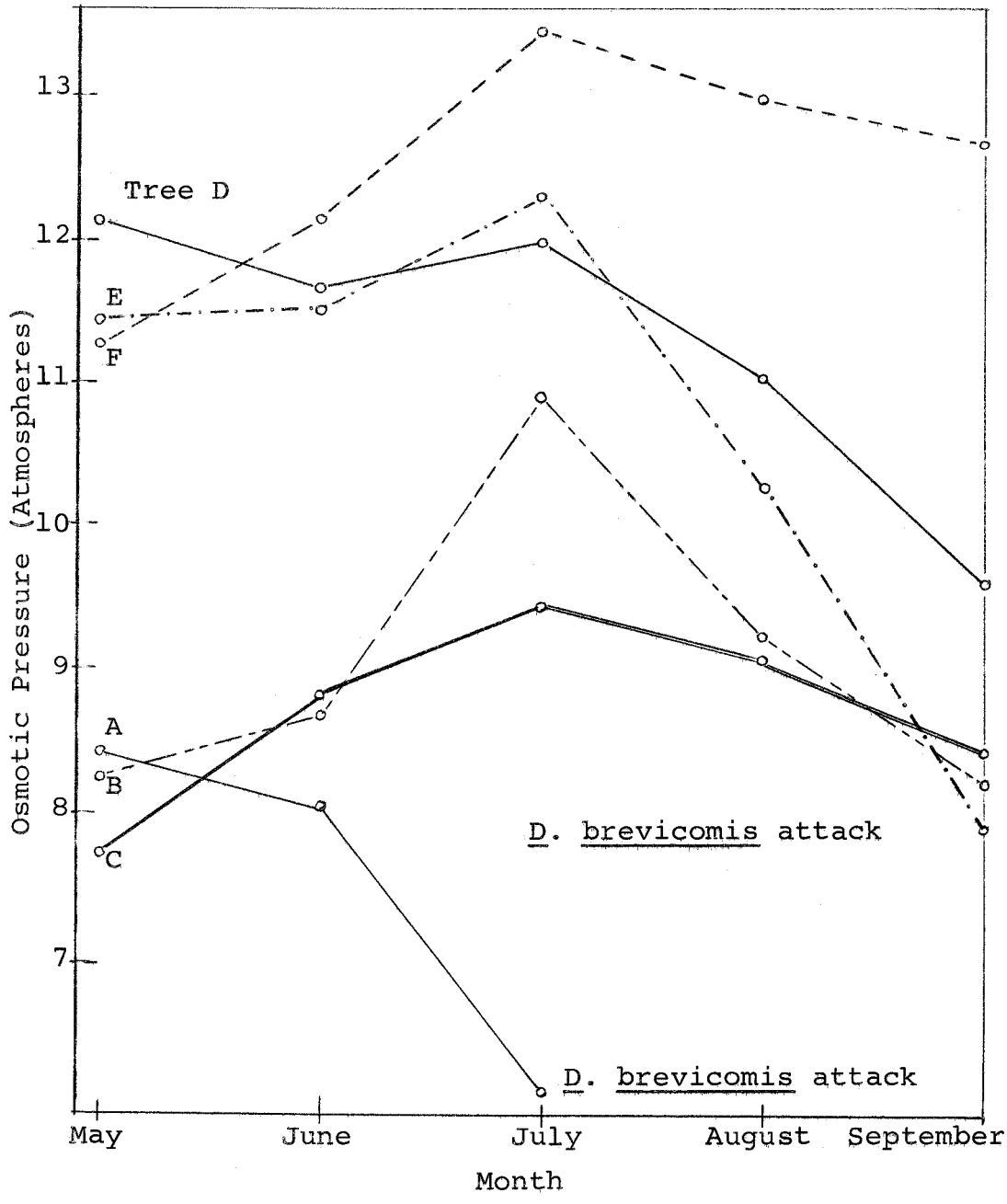

Figure 10. Illustration of osmotic values of expressed phloem sap from six ponderosa pine measured during five different months. Trees A, B and C had zero o.e.p., trees D, E and F had 175, 170, 167 p.s.i. respectively

between the two groups is not maintained. The maximum o.p. potential for the months tested was reached in July. The minimum, neglecting the two trees attacked by Dendroctonus brevicomis, appears to be in September.

The general peak of o.p. exhibited by most members of each group in the month of July is not in accord with the data of Jeffrey (58). While working on the concentration of certain sugars in the phloem of ponderosa pine, he was able to demonstrate a maximum concentration of both reducing and nonreducing sugars in October. Soluble carbohydrates are principally responsible for the o.p. in various trees (36, p.60 and 74). Increases in o.p., however, are sometimes due to a decrease in water content (74, p.87).

Bole and Bharucha (12) failed to demonstrate a direct correlation between water content and o.p. in the older leaves of Avicennia alba Bl. Hepting (53) found the maximum carbohydrate content in the stems of shortleaf pine occurring from April to June, with a slightly less but uniform level from July through September. Worley (145), while studying the carbohydrates of ponderosa pine and Douglas-fir needles, reported an increase of disaccharides in the autumn. Investigation on the mechanisms of the freezing resistance in the needles of ponderosa pine and Psuedotsuga mucronate established a maximum increase in sugar during late

autumn (28). Swiss stone pine (Pinus cembra L.) apparently has a maximum sugar content in January and March (74).

The soluble carbohydrates of ponderosa pine phloem are dominated by sucrose from June through September. Based on a percent dry weight basis total carbohydrates increase throughout the summer (58 and 59). Sucrose is reported as the principal carbohydrate in the inner bark of sugar maple (Acer saccharum Marsh.)(61) and black locust (Robinia pseudoacacia L.)(71, p.112 and 118). Translocation studies on 16 families of trees lead Zimmerman (146) to conclude that sucrose was the predominant soluble carbohydrate and in 11 families was in excess of ten percent of the total carbohydrates.

The failure to detect a marked seasonal increase in sugars which parallel other studies can not be explained. The fact that o.p. values of each group are distinctly different at the initial determination is worthy of attention. This difference, for the most part, is maintained throughout the period of sampling. The obvious implication of these data is that o.e.p. does reflect upon the o.p. It should be pointed out, however, that the number of trees studied is much too small to draw inference to the population.

The effect of sampling, especially on the smaller diameter trees, is an unknown factor. It is felt that

drastic changes may occur when large areas of phloem are removed. After the fourth month of sampling, several of the trees were nearly girdled. As a result of the small number of trees studied and the presumed sampling effect, the fact should be emphasized that the above data are not conclusive though they may be very suggestive.

Phloem thickness. During the course of studies on host differences, the circumstance became apparent that ponderosa pine was characterized by inter- and intra-specific differences in phloem thickness. Trees showing a markedly depressed o.e.p. and o.p. exhibited an inclination toward thin phloem, i.e., $\leq$.050 inches. In addition, phloem tissue appeared to increase in thickness from the butt toward the tree crown.

The investigation of phloem thickness variations was approached by selecting two groups of trees, each of which contained three trees with high o.e.p. and three with low o.e.p. No effort was made in this particular study to relate o.p. and o.e.p. The phloem measurements were obtained from the paper gauge micrometer (Figure 8). The first group of samples was taken at the five-foot level and thereafter at each ten-foot interval; sampling was terminated when the stem diameter reached two and one-half inches. The data for individual trees are given in Table 4.

Table 4. Variation of phloem thickness in ponderosa pine of two o.e.p. categories, i.e., high and low, which were sampled at ten-foot intervals.

Tree	o.e.p. 1/ (p.s.i.)	Height (feet)	Age (years)	d.b.h. (inches)	Sample interval 2/ 3/						
					5	15	25	35	45	55	65
A	1.2	75	106	11.2	.053	.060	.068	.090	.092	.086	.057
B	2	72	96	9.5	.054	.055	.063	.075	.093	.085	-
C	0	65	100	7.5	.038	.045	.056	.063	.049	.040	-
D	150	76	95	11	.066	.072	.086	.098	.107	.104	.097
E	150	75	106	11	.065	.071	.076	.085	.090	.067	.056
F	126	64	101	8	.108	.133	.113	.115	.095	.083	-

1/ Average based on four gauges in trees A-C; based on two gauges in trees D-F.

2/ Intervals in feet.

3/ Measurements are an average of seven samples in thousandths of an inch.

These data are not conclusive, but a pattern of intraspecific difference is somewhat apparent. All trees, with the exception of tree F, exhibited a phloem which progressively increased in thickness from the base to about mid-crown. In this area the trend is reversed and the phloem is characterized by a decrease in thickness. The magnitude of the decrease in phloem dimensions was not followed to the tree apex, but there is no reason not to expect a continual decrease until the meristematic apex is encountered.

When o.e.p. is used as the basis of selection, statements concerning interspecific differences in phloem thickness are unsupportable. The major limitation in constructing a cogent theory is the number of trees studied, coupled with the apparent large diversity between trees of the same o.e.p. category. As an additional aid in portraying tree condition, phloem measurements at breast height were obtained in nearly all investigation concerned with host differences. When these data are included with the above observation it becomes possible to offer a qualified comment on interspecific differences.

The use of phloem thickness as an index of physiological vigor must be tempered with some limitations. As previously stated, a large variance can be expected between groups; a range from approximately .02 inches

to over .2 inches does not appear to be too uncommon. However, measurements taken from breast height do appear to possess some consistency. For the most part, trees exhibiting high o.e.p. and o.p. also can be characterized by thicker phloem than is usually found in trees exhibiting a low o.e.p. and o.p. The use of the comparative is not correct in the definitive sense but it does reflect on the existing difference. The construction of o.e.p. categories with accompanying ranges of phloem thickness is untenable; phloem measurements are felt to be a supplement in portraying the physiological character of a host.

Relative turgidity. The laboratory results in bark and leaf moisture studies on poplar (Populus trichocarpa Torr. and Gray) and willow (Salix sp) have demonstrated excellent correlations between the moisture content of the cells and the level of tree vigor. The term 'vigor' is in reference to such factors as rooting potential and vulnerability to a number of canker diseases (5, 7, 8 and 9). Relative turgidity (r.t.) of functional living cells is an expression of the ratio of fresh weight to total weight after saturation. The procedure is outlined by Bier (5) and the formula can be shown as:

$$\frac{\text{weight of water in bark sample}}{\text{weight of water required to saturate the same sample}} \times 100$$

This procedure includes weighing a fresh sample

followed by a period of oven drying. The sample is then soaked in distilled water until an equilibrium is reached, at which time all surface water is removed with blotting paper.

The results of the above studies indicated that functional tissues with a high r.t. show more capability in resisting disease attack than tissue with a lower moisture content. Bier (6) interpreted this resistance to attack as an expression of vigor and health. The applicability of this approach was examined in the attempt to develop "clinical" indices which accurately portray the physiological vigor of ponderosa pine.

A series of trees, each exhibiting an o.e.p. in excess of 125 p.s.i., were used in the initial phase of this study. A total of ten samples, taken with the 65 mm circular sampler, were used for computing r.t. values. Due to the presence of a moisture gradient from the cambium toward the outerbark, an earlier study (128) found that nine samples with a diameter of 3.0 cm. were required to maintain a standard deviation of 5% or less. Relative turgidity figures were obtained by weighing each phloem sample on an analytical, simi-micro, Mettler H 16 balance. This balance has an accuracy of $\pm$ 0.1 mg and all samples were weighed to the hundredth of a gram. A closer reading was not practical due to the rapid rate of water evaporation from the samples. After weighing,

each sample was placed in a 100 mm x 15 mm petri dish and filled with distilled water. At the end of the 48 hours immersion period, samples were exposed to an oven set at 105°C for approximately 24 hours. Samples were then re-weighed and r.t. values computed.

The results from this study are not shown, but parallel results were obtained in an earlier study (128, p.46). For the most part, r.t. values were in the range from 40-55%. Although there is no reason to expect a similarity between pine and other species, these results are considerably lower than Bier reported for willow (5) and poplar (6 and 7). Vité [1/] maintains that considerable leaching of water soluble materials occurs during the immersion period.

To determine the influence of the distilled water treatment on r.t. values, a large phloem sample (approximately one pound) was taken from a large, fast growing ponderosa pine. The phloem sample was divided into 20 smaller samples, each with a similar fresh weight. From the 20 samples ten were treated in the procedure previously outlined; the remainder were treated in a duplicate manner with the omission of the immersion treatment. Listed summaries of differences in treatments are given in Table 5.

[1/] Personnel communication

Table 5. Effect of a 48 hour immersion in distilled water on freshly removed ponderosa pine phloem

Leached samples			Unleached samples		
Ave.fresh wt. (grams)	Ave.dry wt. (grams)	Ave.wt. lost (grams)	Ave.fresh wt. (grams)	Ave.dry wt. (grams)	Ave.wt. lost (grams)
6.49[1/]	1.56 (1.42-1.69[2/])	4.96 (4.80-5.27)	6.49	2.04 (1.96-2.18)	4.44 (4.31-4.51)
Percent leaching of fresh weight					
23.5[3/]					

1/ All fresh weight samples weighed 6.49 grams.

2/ Range

3/ $\frac{1.56}{2.04} - 100$

The results of this study appear to impart a serious limitation in the applicability of r.t. values as a clinical index. Apparent from Table 5, 23.5% of the fresh weight is lost during the soaking period. This weight loss is presumed to be material composed primarily of soluble carbohydrates and other materials of lesser amounts. The 23.5% weight loss undoubtedly contributes to the low r.t. values, as compared with Bier's results, but this figure will undoubtedly change as the percentage of soluble carbohydrates changes.

The reason for rejecting r.t. as an applicable tool in portraying the physiological character of ponderosa pine is founded on the following reasoning. Based on the assumption that o.p. is an expression, primarily of soluble sugars, then trees exhibiting good vigor, i.e., fast growth, high o.p. and o.e.p., would show a lower r.t. value than trees exhibiting poor vigor, i.e., low o.p. Conversely, Bier's theory of high r.t. values as a reflection of a tree's vigor would have to be held in the reciprocal to be applicable to ponderosa pine. In addition, the range of osmotic pressures that have been found in ponderosa pine would confer such a large range in r.t. values that a definitive classification of vigor types based on this character appears untenable.

Blue-Stain Fungi Studies

The association of bark beetles with blue-stain has been investigated by many workers (15, 16, 33, 44, 50, 54, 55, 72, 93, 94, 97, 112 and 116) but few studies have dealt with the acutal role blue-stain fungi may possess in the colonization of host material. Earlier investigations (107 and 136) concluded that the association was accidental and that the spores of the fungi reach the phloem by a series of fortuitous events. Francke-Grosman (44) felt that the insects are in no way dependent upon the blue-stain fungi; she considers these organisms as commensals. Contrary to this, Craighead (33) suggests a symbiotic relationship in which bark beetles obtain essential nutrients as a result of microorganism metabolism. At any rate, several studies (15, 16, 72 and 116) indicated that a limited activity by blue-stain fungi could rapidly reduce water content and resin flow in coniferous trees and create favorable conditions for beetle development.

An earlier study (132) conducted by Vité and Rudinsky at the B. T. I. Experimental Forest established that ponderosa pine can be successfully infected with blue-stain fungi independent of the beetle. Furthermore, blue-stain fungi penetrated at a rate determined by the physiological condition of the trees as expressed by the o.e.p. However, in accordance with earlier studies (128)

on drastic tree surgery, these inoculations did not seem to influence the overall condition of a tree as long as sufficient inner sapwood was functional for water conduction. In addition, Vité and Rudinsky found that mechanical injury, i.e., removal of bark and phloem from the circumference in 150-centimeter lengths, had little effect on the water conduction in the absence of blue-stain fungi infection. Resin exudation from the xylem surface or blockages of oleoresin penetrations along the periphery of the sapwood prohibited moisture losses. The question remained whether the Ceratocystis infection had an indirect effect on the remaining portion of the tree.

Experiments on standing trees. To further investigate the question of Ceractocystis infections, o.e.p. values were determined for 40 approximately 30-year-old ponderosa pine, 19-23 cm. d.b.h. and averaging nearly ten meters in height. From these 40 trees, seven were chosen according to their diurnal o.e.p. pattern and placed in one of three arbitrarily selected categories, e.g., high pressure 110-158 p.s.i., medium pressure 21-90 p.s.i. and low pressure 0-20 p.s.i. Individual treatments within each category consisted of a 40-centimeter-wide inoculation (breast height) on the whole circumference using C. ips as the inoculum and basal applications of herbicide. Other trees were felled as control material for brood development. After four days, six males of

Ips confusus were introduced above and below the inoculation band. This procedure was repeated each week for four weeks. With the exception of the felled trees, o.e.p. measurements were taken two and three weeks after the first attempt to introduce beetles.

In trees inoculated with blue-stain fungus, Ips introductions were not successful nor were significant changes noticed in the o.e.p. during the first four weeks of the experiments. The o.e.p. in the two trees treated with herbicide had been reduced to zero two weeks after the first attempt to introduce Ips males. Four of the 12 males introduced at this time were successful and within three days both trees were sustaining mass attacks by the field Ips population. This period between the first successful attack and the subsequent mass attack is in agreement with earlier observations (144). A similar attack pattern was noted for the felled trees.

Approximately 45 days after the mass attack, herbicide treated trees were felled and the brood development compared with that of the trees felled at the onset of this experiment. Although enumerated data were not taken, an observational comparison was accomplished as a result of the apparent similarity in attack density. As a consequence of these observations, differences in brood development were noted between the two types of trees. The brood in the standing trees developed at a

slower rate than the felled trees. This retardation in development can be attributed to a deficiency in host conditions, i.e., nutrition, moisture level, etc., or it may be accounted for as a result of the herbicide treatment. Emergence records on caged logs taken from a felled tree indicated maximum beetle emergence 30-35 days after the initial attack, while brood was still present in the standing trees 48 days after the initial attack.

Approximately two months after the inocula treatments, two-inch discs removed from the center of the 40-centimeter-wide inoculation showed that the development of blue-stain fungi was markedly affected by o.e.p. This observation was re-enforced experimentally by reducing the o.e.p. in trees of normal exudation pressure by spraying the base with oil and herbicide. In these trees the blue-stain fungus penetrated the sapwood readily in contrast to similar trees which had not been treated (Figure 11). The outer xylem of herbicide-treated trees was so effectively saturated with oleoresin that blue-stain development was not detected. In comparison, trees of medium and low o.e.p. showed less blockages by resin, and blue-stain was apparent to some degree.

Differences in blue-stain fungi development were found between trees with a low o.e.p. and trees which had been artificially reduced to a low o.e.p. The sapwood in the herbicide-treated trees was extensively stained,

Figure 11. The oleoresin exudation of tree A effectively prohibited blue-stain penetration through resin blockages in the outer xylem (arrows). Tree B, which had been experimentally reduced to zero p.s.i., through a basal treatment of oil and herbicide, was characterized by an extensive blue-stain fungus permeation (arrows).

suggesting a more favorable media for blue-stain fungi development. The fact that blue-stain fungi is restricted to a certain sapwood moisture has been established through numerous workers (10, 30 and 93). It therefore seems plausible to suggest that the poor moisture relations of low o.e.p. trees retarded the rate of blue-stain penetration.

Experiments on cut trees. The experiments on live trees confirmed that the effect of Ceratocystis ips is restricted to the locality where the actual infection took place. To consider whether there are side effects, such as accelerating the moisture loss and discoloration of trees due to successful blue-stain infection, additional experiments were performed on cut trees. Seven 15-year-old ponderosa pine, 10-15 centimeters d.b.h., averaging approximately six meters in height, were felled and suspended by ropes and pulleys. Percent moisture content of the current year's needles on the lower crown portion was determined gravemetrically and used as the indicator of the water reserve in the tree. The various treatments are as follows:

Tree Number	Treatment
1	Bark and phloem removed in a two meters length from the whole circumference of the tree base; exposed xylem wrapped with moist cellucotton and covered with polyethylene sheeting.
2	Bark and phloem removed in a two meters length from the circumference at the tree base; exposed xylem covered with masticated phloem from Ips-infested logs and covered with polyethylene sheeting.
3	Forty-five centimeter area above root collar sprayed with herbicide two days prior to felling; treated area of bole removed before tree was suspended.
4	Bark and phloem removed in a 40 centimeter length from circumference just below the first living limb stem removed .5 meters below inoculation band; exposed xylem wrapped with masticated phloem from Ips-infested logs, area covered with polyethylene sheeting.
5	Stem removed just below first living limb.
6	Bark and phloem removed from the circumference just below the first living limb to tree base.
7	Bark and phloem removed in a two meter length from the circumference at the tree base; exposed xylem wrapped with cellucotton moistened with 1375 mls of pure C. ips culture, covered with polyethylene sheeting.

Introduction of blue-stain fungi by attacking beetles was reduced by spraying each tree with a 50% wettable

powder DDT solution. Figures 12 and 13 indicate that Ceratocystis ips plays little or no part in needle moisture loss in cut trees. A 20-centimeter disc was removed from the inoculation band of tree 7 every three days. Within 13 days the visible blue-stain had penetrated approximately two-thirds of the sapwood; however, 14 days after the inoculation the needle moisture was only reduced to 91 percent. In contrast, tree 6 displayed an 18 percent needle moisture content after only six days. Similarities in moisture loss patterns in needles of trees 4 and 5 are probably a result of nearly equal stem volumes. As a similar corollary, trees 3 and 1 are characterized by nearly equal stem dimension. The variance from the above explanation, as reflected by tree 2, cannot be explained.

The limited results in this study are in accord with an earlier study (132) in which the fact was demonstrated that infection of ponderosa pine depends upon a subnormal physiological condition of the host tree. A similar requisite has been found for successful bark beetle infestations (144). Only trees of low o.e.p. were unable to prevent the penetration of blue-stain fungi through resinous blockages in the outer sapwood. In contrast, trees of normal physiological condition as expressed by high o.e.p. apparently limit the blue-stain to the exposed xylem.

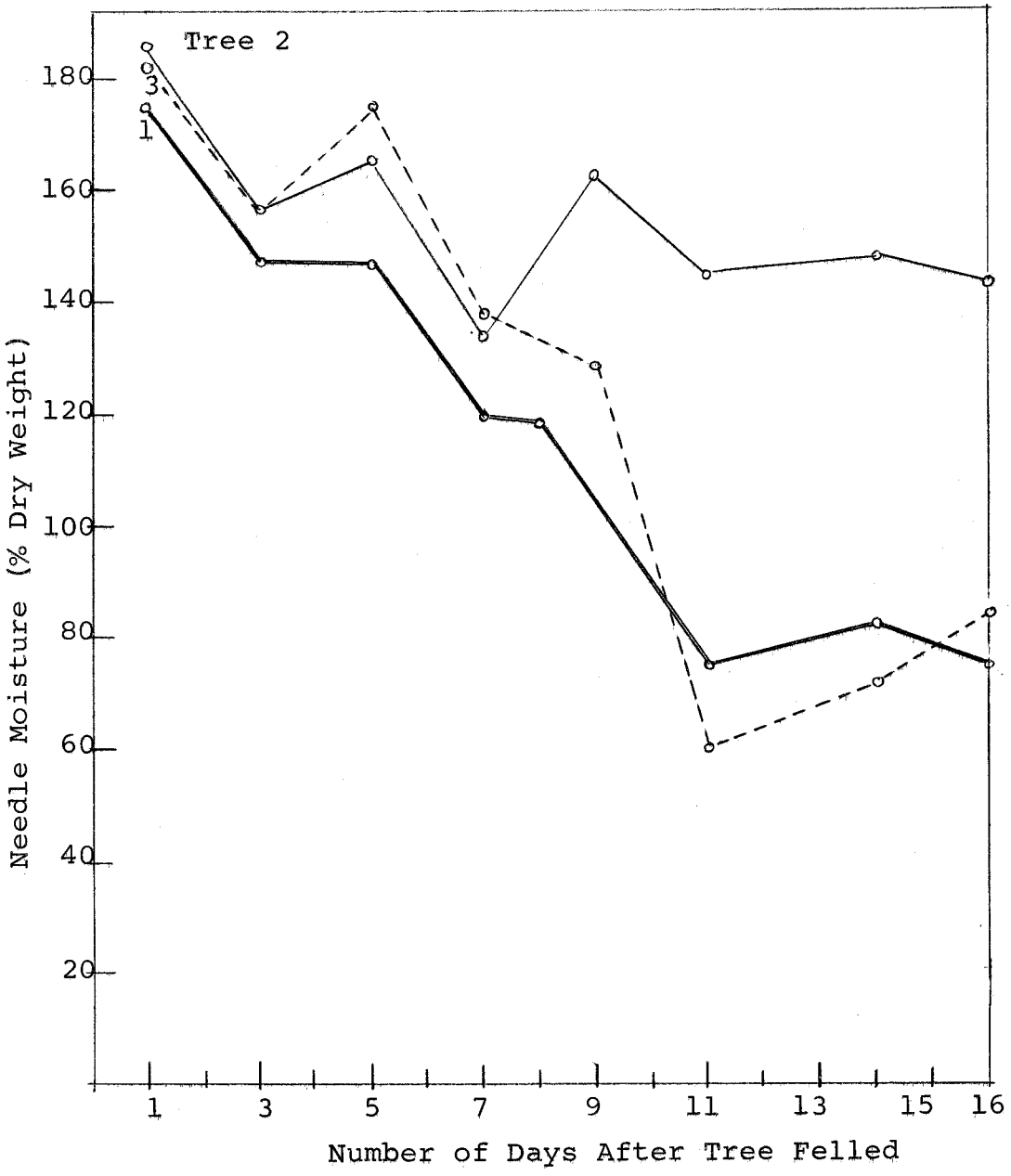

Figure 12. Effect of three inoculation treatments on cut ponderosa pine trees reflected by moisture loss from the current year's needles on the lower crown portion

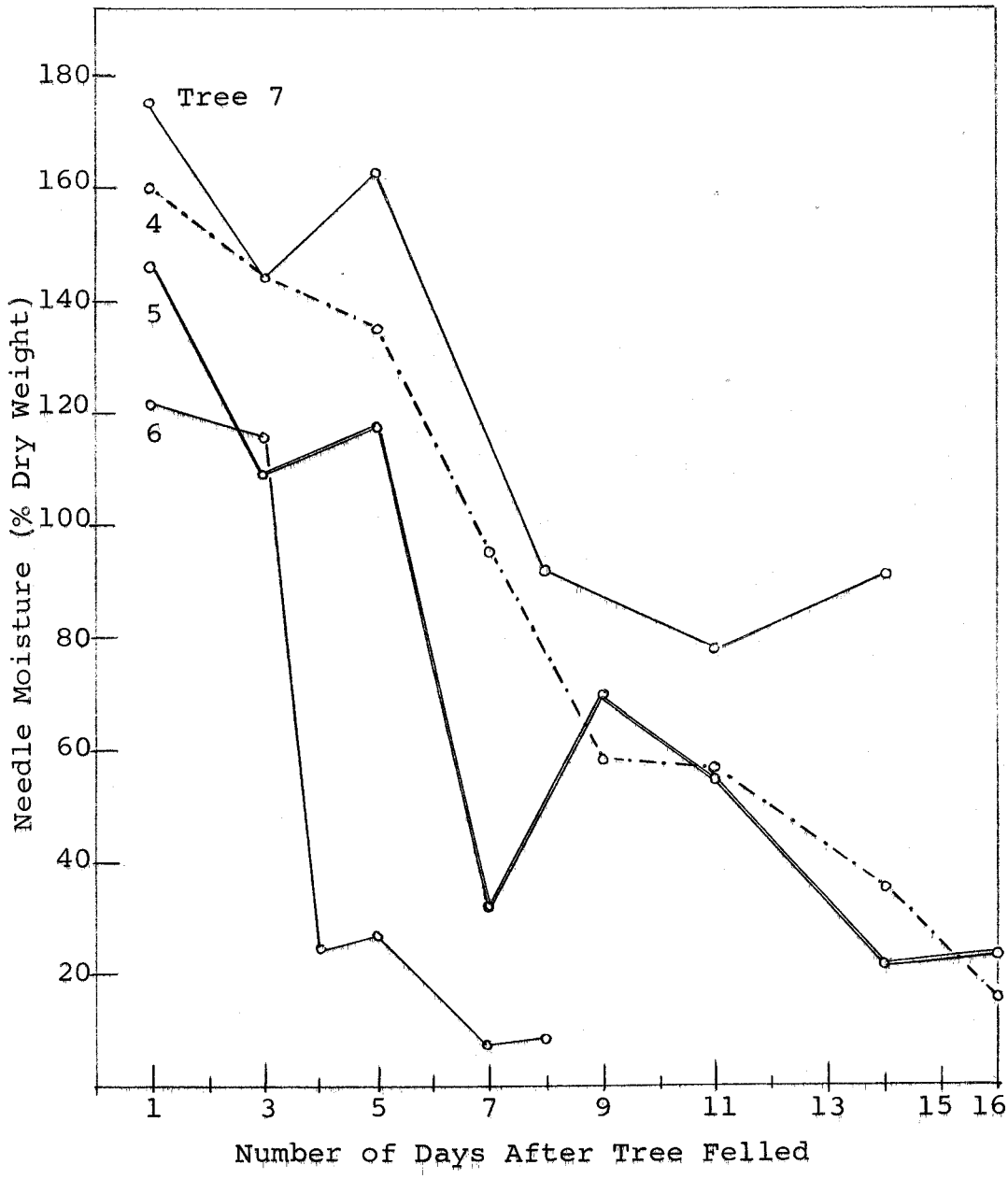

Figure 13. Effect of four inoculation treatments on cut ponderosa pine trees reflected by moisture loss from the current year's needles on the lower crown portion

The experiments on cut trees suggest that the effect of blue-stain fungi are limited to the actual extent of each infection. A similar conclusion was reached by other workers (16) while studying the relationships between D. frontalis and Ceratocystis species. Contrary to one theory (33), a blue-stain fungi infection does not appear to have any distinct influence on the physiological condition of the host. When cut trees are compared with trees infested with bark beetles the color change of foliage does not appear to be accelerated by the fungal infection; instead the change can be attributed to the girdling or debarking effect of the attacking insects. This conclusion is in agreement with the field observations that trees with an uninfested crown, but infested lower bole, do show foliage discoloration much later than trees infested in the crown area.

Influence of Relative Humidity

Dethier (34, p.164), in considering conditions which affect thresholds of response, stated, "In olfactometers three of the most important factors tending to influence chemotatic behavior are temperature, humidity, and air movement." Frankel and Gunn (42, p.288-289), after reviewing the literature on moisture and its effect on arthropods, concluded that humidity is presented as a concentration of water molecules, and therefore should be

regarded as a kind of chemical stimulus. Because humidity is attractive to many arthropods and repellent to others (34, p.14), several workers (42, 66, 73, 79, 100, 101 and 125) have been led to study the effect of moisture on arthropod behavior patterns. In reflecting upon the influence of temperature, humidity and air movement, Dethier (34, p.164) concludes with the germane statement, "By affecting the over-all behavior of an insect these factors cause a variation in response that may incorrectly be interpreted as a modification of threshold." The presence of a possible hygrostimuli from materials in threshold studies prompted a series of tests on the moisture concentration of the air stream passing through the laboratory olfactometers.

The moisture concentration of the ventilating air stream which minimized the preference for various humidity gradients was determined in the following manner. Nine small jars containing pieces of paper towel saturated with distilled water, a solution of sodium chloride and anhydrous calcium chloride, were affixed to the pegboard olfactometer (Figure 3) and arranged so that a discontinuous sequence was obtained. Empty jars were included as the checks and incorporated into the treatment placement in a similar fashion. Although measurements were not made, the data of these tests are based on the assumption that materials of these types form a

moisture gradient on the arena surface. The ventilation stream's humidity was regulated by forcing the air through foam rubber saturated (100% R.H.) with distilled water and a one-inch barrier of anhydrous calcium chloride (0% R.H.). The ambient humidity (38% R.H.) was obtained by removing the above mentioned barriers.

The results of these tests are given in Figure 14 and Appendixes I and II. The intensity of preference is based on the following computation:

$$\frac{R-NR}{T} \times 100 \qquad (100)$$

R = numbers of beetles responding, NR = numbers of beetles not responding, and T = total number of beetles used in each test. Intensity of preference is a subjective description of the reaction of Ips confusus when presented to a humidity gradient, but it does serve to illustrate the general effect of modifying the olfactometer air stream. All plotted points are based on five replications using 100 female Ips confusus for each replication.

When the air stream is saturated with water vapor, Ips confusus females show no preference to moisture gradients and it is obvious from Figure 14 and Table 6 that a preference for moisture gradients were not present. Whereas significant differences between saturated paper towels and anhydrous calcium chloride were evident in air streams of 38 percent and 0 percent

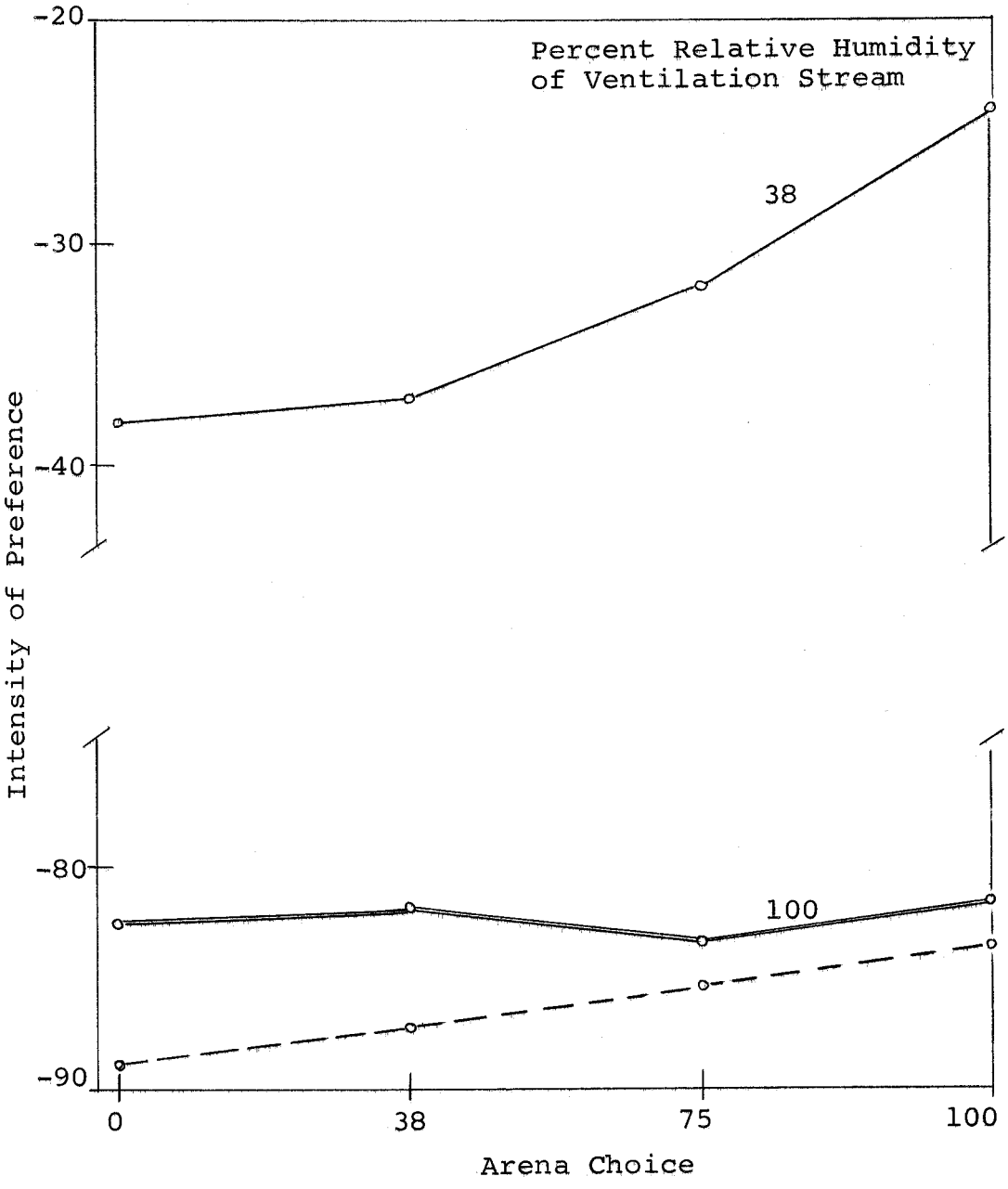

Figure 14. Preference by Ips confusus for various humidity gradients when tested in olfactometers with ventilation streams of 0%, 38% and 100% relative humidities

Table 6. Summary of the comparative preference by female Ips confusus for four humidities in laboratory olfactometers with ventilation streams of 100, 38, and 0 percent relative humidity

Olfactometer ventilation stream	Moisture gradient (percent R.H.)	Total number of beetles responding	Percent of total (approx.)	Mean number of beetles per observation	Total number of observations per factor
100% R.H.	100[1/]	21	4.2	1.4	15
	75	13	2.6	.9	15
	38	20	4.0	1.3	15
	0	17	3.4	1.1	15
38% R.H.	100[2/]	111	41.2	6.2	18
	75	71	26.4	3.9	18
	38	46	17.1	2.5	18
	0	41	15.2	2.3	18
0% R.H.	100[1/]	27	5.4	1.8	15
	75	18	3.6	1.2	15
	38	9	1.8	.6	15
	0	1	.2	.1	15

[1/] Test composed of five replications with 100 beetles per replication.

[2/] Test composed of six replications with 100 beetles per replication.

relative humidity. An analysis of variance test was not applied to the olfactometer with a saturated stream; however, the statistical analysis indicates that the differences were significant at the .5 percent confidence level (Appendixes I and II) for air streams of 38 percent and 0 percent respectively. The anomaly of the data recorded in the olfactometer with a ventilation stream modified to 38 percent is not apparent but may be due to the age of beetles tested. The beetles used in this test were nearly eight days older than the beetles used in the other experiments. This could account for the high level of preference for all moisture gradients as well as the magnitude of fluctuation. Perttunen (98) investigated the humidity preference of eight species of carabid beetles. Differences in preference were demonstrated for some species according to the degree of desiccation. The common earwig (Forficula auricularia L.) is known to change its preference for humidity levels according to the season. Earwigs collected in the summer prefer the dry alternative but concentrate on the moist alternative after being subjected to periods of desiccation (99). Drosophila melanogaster apparently prefer higher humidities when specimens are desiccated (102).

Ips confusus show a preference for high moisture gradients when the ventilating stream of a laboratory olfactometer is low. This preference would interfere

with thresholds or comparative responses to different substances; consequently all laboratory olfactometers were adjusted to impose a saturated state on the ventilating air stream.

The data from the preceding experiments are conclusive in that moisture levels of the ventilating stream influence the response pattern of Ips confusus. This conclusion was based on the apparent preference that beetles show for certain humidity gradients in the absence of the attractive principle. It seemed essential to determine if a similar preference would become evident in the presence of Ips confusus' pheromone.

In order to determine if Ips confusus manifested a different pattern than was described in above-mentioned experiments, a study was designed which would test the effect of various air streams in the presence of the attractant principle. Similar conditions were imposed on the air streams of the laboratory olfactometers. The humidities were 100 percent, 0 percent, and room ambient (35 percent) in olfactometers 1, 2 and 3 respectively. The olfactometers were in tandem (Figure 2) and all experimentation was conducted at 23 $\pm$ 2^{o}C. Treatment placement beneath each olfactometer arena was similar to the method previously described, i.e., no two treatments occurred in unison. Treatments consisted of 0 and 100 percent humidity gradients and boring dust from two

trees with o.e.p. in excess of 129 p.s.i. The attractive principle was obtained by forcing 20 male Ips confusus into nine 3-1/2 inch discs taken from each tree. The beetles were permitted to feed for 72 hours at 23 ± 2°C. Boring dust was collected by inverting the discs over a small funnel which emptied into dixie cups. Frass from both trees was tested in each olfactometer.

The results shown in Figure 15 and Appendix III are based upon three replications of 100 female beetles each. All statistical calculations are based on the number of beetles responding to each treatment; therefore, statistical differences are comparisons of individual treatments within each olfactometer. As indicated in the discussion on "statistical analysis," all F values in the factorial analysis of variance are obtained by considering replications as a fixed factor and utilizing the residual value for the denominator.

The graphic representation of the data (Figure 15) recorded in this experiment support the earlier conclusion that the moisture level of the ventilation stream is an important variable in laboratory olfactometers. When the ambient humidity is low, female Ips confusus prefer high moisture gradients. The presence of the species pheromone does not appear to influence this pattern, but absolute values of preference are of course altered. This is evident when 100 percent and 0 percent moisture

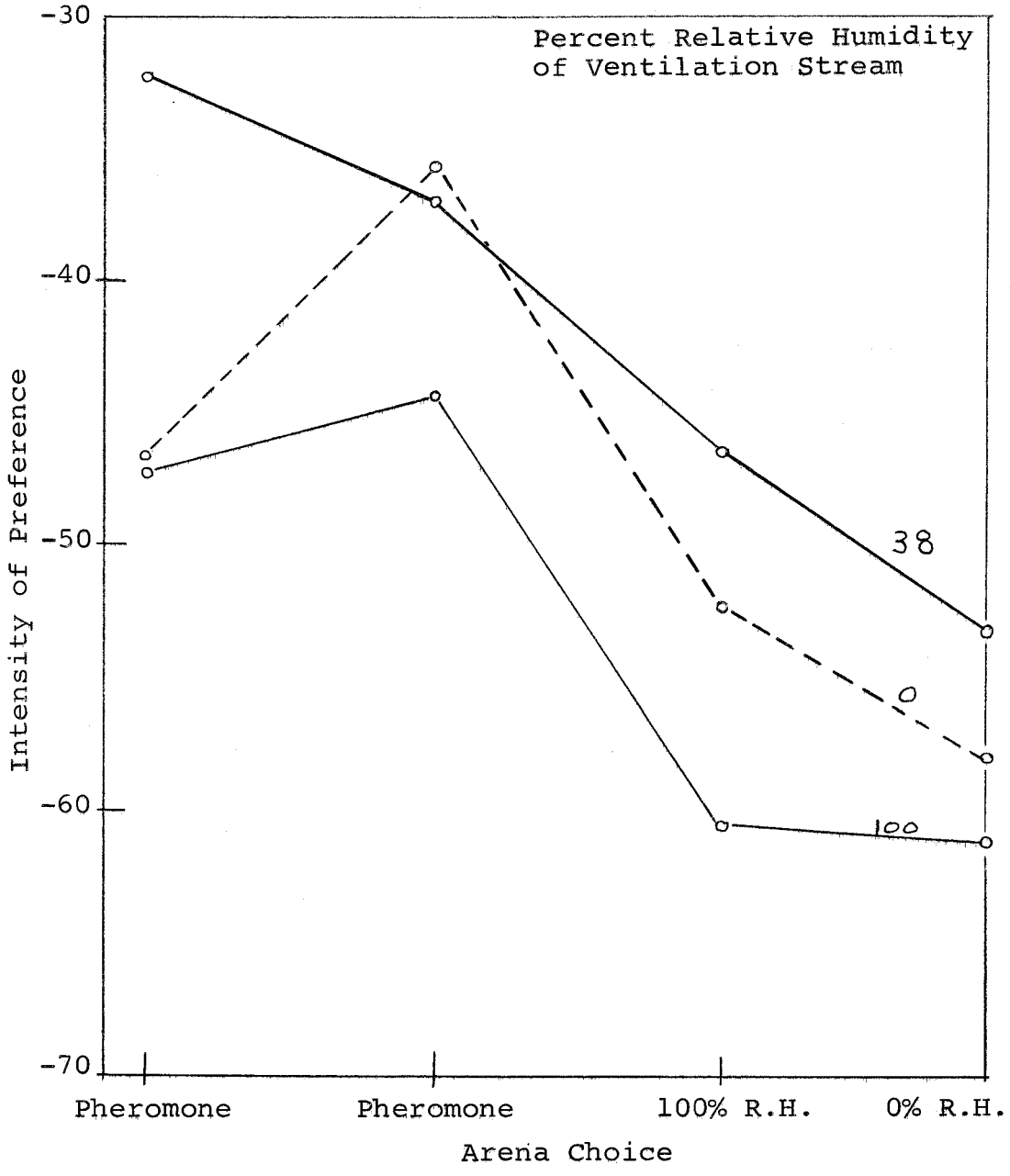

Figure 15. Preference by Ips confusus for various humidity gradients when tested in the presence of the species-specific pheromone and ventilation streams with three different relative humidities

gradients are compared in olfactometers with a ventilating stream of 100 percent R.H. (Figures 14 and 15). The degree of preference is less in the previous experiment than shown in the latter one, but significant differences between 100 percent and 0 percent are not found in either of the tests.

The factorial analysis of variance could not detect a significant difference between the means of the total number of beetles responding in each olfactometer, regardless of the air stream characteristic. A difference in treatment means significant at the .5 percent level was found, but this is not surprising when the nature of the materials being tested are considered. The pheromone would be expected to elicit a higher response than anhydrous calcium chloride. As mentioned in the section on "statistical analysis," treatment placement resulted in the circle of small jars being divided into three or four sectors depending on the number of treatments. The sector effect, Table 7, suggests that released beetles prefer one portion of the arena surface over others. This particular set of experiments indicated treatments in sector one were more efficient in capturing female Ips confusus. The treatments are numbered 1 through 12 with the first treatment being placed in the jar furthest from the olfactometer door. Commencing in a clockwise fashion, the next four treatments would be

included in sector 1.

Table 7. Summary of numerical data recorded from female Ips confusus' preference to various materials in laboratory olfactometers with air streams of 100 percent, 35 percent, 0 percent relative humidity

Main factor	Total number of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Olfactometer				
1 (0% R.H.)	110	29.5	2.3	48
2 (38% R.H.)	138	37.0	2.9	48
3 (100% R.H.)	125	34.0	2.6	48
Treatment				
Pheromone[1/]	315	84.5	7.4	36
100% R.H.	49	13.1	1.8	27
0% R.H.	9	2.4	.1	27
Sectors				
1	165	44.2	4.6	36
2	130	34.8	3.6	36
3	78	20.9	2.2	36

[1/] Includes totals from both trees

The preference for high moisture gradients when the ambient humidity is low appears to be a characteristic of female Ips confusus. This pattern of behavior is not altered through the presence of the attractant principle.

To minimize the interfering effect of moisture preferences it seems tenable to suggest that all laboratory studies concerned with olfactory responses be conducted in a water saturated atmosphere.

Olfactory Studies In Vitro of Dietary Carbohydrates

In terms of breeding material selection and certain other behavioral attributes, spring and fall populations of Ips confusus are distinctly different (48, 51, 124, 140 and 143). In the spring Ips confusus select slash and other host debri as breeding material; however, in the late summer and fall young, apparently fast growing trees of less than 12 inches and tops of larger trees with a d.b.h. in excess of 24 inches are attacked (51 and 124). Comparative response studies (48) of a single brood demonstrated that early emerging beetles respond in numbers at a distance of five meters nearly five times greater than beetles emerging approximately 14 days later. Other studies (142 and 143) have found a continual lowering of the level of attraction of male frass as the season progressed. Chapman (20) concluded that Trypodendron vary in their flight capacity and spring broods easily initiate flight while the converse is true for fall broods.

Seasonal changes in host physiology, particularly carbohydrate concentrations, are well documented

(28, 58, 59, 95 and 145). The literature pertaining to results of nutrition studies on phytopoghous insect has been reviewed by Lipke and Fraenkel (78); for example, Pea aphid exhibits lower reproduction rates on plants deficient in calcium, magnesium, nitrogen, phosphorus and potassium. Red scale is retarded on lemons when fertilized with nitrogen. Wellington (137) mentions that changes in plant quality, as a consequence of climatic changes, do affect the insect parasite. Chararas (27) and Wood and Bushing (143) suggest that carbohydrates may play an essential role in some beetles' biology. The former author found that glucose, sucrose, arabinose and stachyose favor development of the weevil Pissodes notatus F. The latter authors suggest that differences in behavior and physiology of Ips confusus may be related to seasonal carbohydrate changes.

The role of various sugars as possible substrates of attractants has been the principle of many olfactory studies. These studies were concerned, primarily, with various carbohydrates as fermentation substrates for microorganisms rather than a nutritional moiety essential to its biological synthesis as suggested by Wood and Bushing. Dethier (34, p.75-102) presented an extensive coverage of the fermentation products (alcohols, acids, aldehydes and esters) of the major groups of plant carbohydrates. In that sugars have no vapor pressure,

past studies (36, 59 and 82) were unable to detect a response to sugar per se. Person (97), in proposing his classical theory on host selection by Dendroctonus brevicomis, implicated the products of yeast fermentation as the source of the secondary attractant. A later study (117) failed to demonstrate that yeast isolates from Dendroctonus and Ips species were capable of fermenting sugars except glucose, and then even the fermentation of glucose was weak or negative.

Literature heretofore reviewed on o.p. studies, seasonal changes in host physiology, and seasonal changes in habits and other attributes of Ips confusus behavior prompted the initiation of a series of nutritional studies. The point was reasoned that a supplementary addition of carbohydrates to homogenized phloem containing male Ips confusus may result in a higher level of pheromone production. It seemed reasonable to expect that one or more sugars may be responsible for increasing the apparent level of pheromone synthesis.

Preparation of the ground phloem was according to the method described by Bedard (4). Briefly, freshly removed phloem is homogenized in a food blender until the tissue is reduced to a consistent pulp. Excess water is drained off and the pulped phloem is placed in the small jars affixed to the bottom of the olfactometer arena. The final consistency and moisture level in the

jars were obtained by firmly pressing the pulp with dry paper towels. All carbohydrates were tested by adding 1.5 milliliters of a 1 molar concentration to approximately 8.9 grams of pulp. Ten male Ips confusus were forced into the pulp in each jar and allowed to feed for 72 hours at room temperature (24 ± 4°C.). Laboratory olfactometers with water saturated ventilation streams were used throughout these studies.

The test was implemented by using reagent fructose, maltose and sucrose in laboratory olfactometer number two (Figure 2). Although maltose is not a major constituent of ponderosa pine phloem, Vité[1/] was able to elicit an increase in response by female beetles to frass produced by male Ips confusus feeding in homogenized phloem supplemented with maltose. The above mentioned carbohydrates were tested in an identical manner but in different seasons of the year. The first test was completed on April 17, 1963 and the second on August 29, 1963. The phloem for both tests was taken from the same tree distinguished by a d.b.h. of 14 inches, an age of 60 years and an o.e.p. in excess of 118 p.s.i. on April 15, 1963. The results are based on nine replications per test with 100 female Ips confusus per replication.

The summary of results for the two experiments are given in Table 8. The data support the conclusion that

1/ Personnel communication

Table 8. Summary of the comparative response by female Ips confusus elicited by males feeding in homogenized phloem supplemented with three different sugars, i.e., fructose, maltose and sucrose

Test	Main factor	Total number of beetles responding[1]	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
1	Treatment				
	Control[2]	115	19.2	4.3	27
	Fructose	110	18.3	4.1	27
	Maltose	183	30.5	6.8	27
	Sucrose	192	32.0	7.1	27
	Sector				
	1	236	39.3	6.5	36
	2	213	35.5	5.9	36
	3	131	25.2	4.2	36
2	Treatment				
	Control[2]	71	20.1	2.6	27
	Fructose	58	16.4	2.1	27
	Maltose	105	29.7	3.8	27
	Sucrose	119	33.7	4.4	27
	Sector				
	1	119	33.7	3.3	36
	2	158	44.7	4.4	36
	3	76	21.5	2.1	36

[1] Test composed of nine replications with 100 beetles per replication.

[2] No carbohydrate added.

the additon of sucrose or maltose to homogenized phloem results in an increase in response which is significantly better at the .5% level (Appendixes IV and V) than phloem supplemented with fructose. The fact that the fructose mean is consistently lower than the control mean is worthy of consideration; however, a significant difference can not be demonstrated between the two treatments. The tendency of this sugar to reduce feeding and/or pheromone synthesis cannot be explained. Parker (95) found fructose at 0.4 and 0.5% (fresh weight) during February and May respectively in the living bark of ponderosa pine. In addition, it appears important to note the pattern of response as elicited by male beetles fed supplementary carbohydrates. This pattern is easily demonstrated by transforming the data into intensity of response values (Figure 16). Although the absolute values between the two tests are different, the relative effects within the tests are similar.

The peculiarity of the response patterns as measured by the females' avoidance of one sector on the arena surface is again evident. As demonstrated by Tables 7 and 8, sector 3 apparently manifests properties which are not conducive for species specific pheromone eliciting a comparable olfactory stimuli. The treatment sector interaction in test 2 was a result of the very low number of females which responded to the fructose treatment in sector 2. An examination of the number of

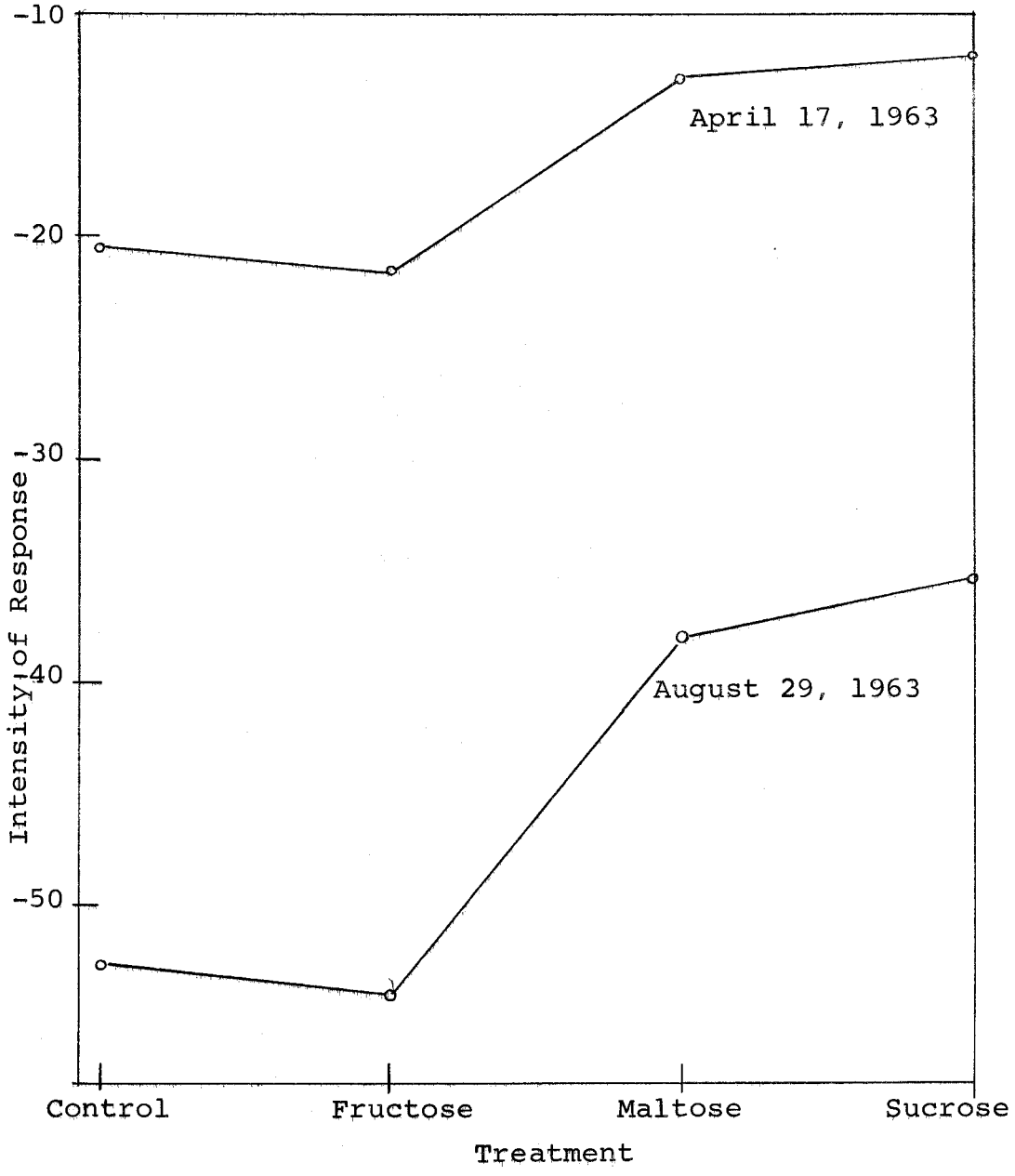

Figure 16. Effect of various carbohydrate supplements to the diet of male Ips confusus as determined by dissimilitudes in response patterns of female Ips confusus

males successfully feeding in this treatment failed to explain the low response.

The results of these tests suggest that addition of maltose and sucrose to the diet of male Ips confusus affects their ability to produce the attractive principle. Although the addition of maltose and sucrose apparently favors the pheromone synthesis, the question still remained if these sugars were filling a dietary deficiency or if the increased pheromone "concentration" was simply a coincidence and not a true treatment effect. Furthermore, if the results were a treatment effect, then the influence of other sugars normally found as major phloem constituents may also be a requisite dietary component for pheromone synthesis.

To examine the above considerations, a series of tests were performed using potato starch, glucose and raffinose. The selection of these carbohydrates was based on the results of Parker (95). With the exception of potato starch, Parker reported that the latter sugars are major constituents of ponderosa pine phloem. The remaining studies concerned with carbohydrate supplements were performed in the exact manner as described for the preceding set of experiments.

Based on the fact that maltose usually occurs in lower concentrations than sucrose (71, p.99), plus the fact that Parker did not detect maltose in his

chromatographs, sucrose was selected from the preceding experiments for continued comparisons. The second set of comparisons included potato starch, glucose and sucrose. Due to the fact that a portion of the starch did not dissolve in the distilled water the conecntration of starch was approximately five percent (w/w). In addition to carbohydrate comparisons, the experiment was designed to re-enforce the data on the influence of relative humidities within the olfactometers. Consequently, olfactometers 1 and 2 were utilized with an air stream of 0 percent and 100 percent respectively.

The information from these tests on the relative humidity of the air streams support the data given in Tables 7 and 8. In terms of total numbers of beetles responding, a statistical difference was not evident between the two olfactometers (Appendix VI). Apparently the humidity of the air stream has little or no effect on the absolute level of response.

As evident from Table 9 the addition of carbohydrates failed to elicit marked changes in numbers of female Ips confusus responding to the various treatments. In opposition to the results present in Table 8, sucrose treatments did not elicit an increase in the numbers of responding female beetles. This low level is explained in part by the sector-treatment interaction (Appendix VI). Beetles responding to sucrose treatments in

Table 9. Summary of the comparative response by female Ips confusus elicited by males feeding in homogenized phloem supplemented with three different carbohydrates, e.g., starch, glucose and sucrose

Main factor	Total number 1/ of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Olfactometer 1 (0% R.H.)	412	82.3	6.9	60
Olfactometer 2 (100% R.H.)	423	84.6	7.0	60
Treatment 2/				
Control 3/	214	21.4	7.1	30
Starch	174	17.4	5.8	30
Glucose	254	25.4	8.5	30
Sucrose	193	19.3	6.4	30
Sector 2/				
1	312	31.2	7.8	40
2	220	22.0	5.5	40
3	303	30.3	7.6	40

1/ Test composed of five replications with 100 beetles per replication.

2/ Represents totals from olfactometers 1 and 2.

3/ No carbohydrate added.

sector 2 were approximately four times less than the remaining two sectors. This interaction probably accounts for a portion of the low response; however, the alternative must be considered. The difference between the response mean of the control and the response mean of the sucrose supplement is statistically inseparable. This fact enforces the conjecture that treatment effects are coincidental and not related to a dietary deficiency. A statistical significant increase (at 1% level) in numbers of females responding to the glucose supplement, as compared to starch supplement, supports the contention that sugars are effectual as a dietary supplement. These conflicting data preclude a knowledgeable conclusion concerning the role of dietary sugar. As a corollary, an additional experiment was performed which compared glucose, sucrose and raffinose against an unsupplemented control.

A highly significant difference between the control mean and glucose supplement mean is evident in Table 10 and Appendix VII. These results indicate that glucose supplements favor pheromone synthesis. Based on this single test, raffinose and sucrose appear similar in their effectiveness as a dietary supplement. Of interest was the fact that sucrose treatments elicited a greater response than controls. This is in accord with the first carbohydrate study (Table 8, Appendixes

Table 10. Summary of the comparative response by females elicited by males feeding in homogenized phloem supplemented with three different sugars, e.g., raffinose, glucose and sucrose

Main factor	Total number 1/ of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Treatments				
Control 2/	55	16.0	3.0	18
Raffinose	70	20.4	3.9	18
Glucose	141	41.1	7.8	18
Sucrose	77	22.4	4.3	18
Sectors				
1	175	51.0	7.3	24
2	103	30.0	4.3	24
3	65	18.9	2.7	24

1/ Test composed of six replications with 100 beetles per replication

2/ No carbohydrates added

IV and V), but inconsistent with the data of the preceding study (Table 9, Appendix VI). As apparent from earlier studies, sector 3 was ineffectual in trapping responding beetles. This characteristic of olfactometer 2 cannot be explained at this time. The replication-sector interaction is a result of an increase in response to sector 1 on the sixth replication. No biological significance is

attached to this observation. The control sector interaction is a result of low level response to the control jar in sector 3. In light of the sector effect, it is difficult to determine if the low response is a result of the sector or some characteristic of the treatment. The usual number of beetles (6-10) were feeding on the phloem homogenate and apparently producing frass.

As a result of the four separate experiments, with a numerical summary of data presented in Tables 8, 9 and 10, it seems tenable to conclude that certain dietary carbohydrates can influence the pheromone synthesis. The carbohydrates raffinose, potato starch and fructose appear ineffectual in increasing the attractant level. Parker (95) was unable to detect raffinose during May as a constituent of the living bark of ponderosa pine; however, this trisaccharide was found in the living bark during February but at a very low concentration (0.2% fresh weight). The fact that dietary potato starch does not enhance the level of response is of interest. The data presented in Table 8 indicate that maltose is effective as a dietary supplement. The partial hydrolysis of starch by beta-amylase yields largely maltose (37, p.141). With the exception of a few species, e.g., Popilla amylase is found in Coleoptera (138, p.331). Therefore, it becomes difficult to explain the apparent failure of starch to enhance the pheromone level, unless

Ips confusus do not possess the system of starch splitting enzymes.

The data conclusively implicate glucose as the most effective dietary carbohydrate tested. In all tests, Tables 9 and 10, a dietary supplement of glucose enhanced the level of response. Raffinose is seemingly equal in efficiency to sucrose in pheromone enhancement when used as a dietary supplement. Both sugars have a single glucose moiety in their respective molecule (37, p.133). The monosaccharide fructose, lacking a glucose moiety, appears to be neutral as a dietary supplement.

Although the preceding data are suggestive in implicating certain carbohydrates in pheromone activity, they are by no way conclusive. The inconsistency of the data was sufficient to warrant the continuation of the dietary studies but with a different approach. The point was reasoned that an unknown fat-soluble substance may remain in the phloem after the homogenizing step. As a consequence of this conjecture a series of experiments were initiated in which the homogenized phloem was given an additional treatment with one of several organic solvents.

The first test was implemented by removing a large piece of phloem from a mature ponderosa pine. Particular attributes such as age, height and o.e.p. were not recorded. The tissue was removed immediately and

stored at -24°C. for several days. The sieve elements of phloem are considered to be specialized parenchyma and, therefore, enclosed by a plasmodesmata (39). Consequently, the ice crystals resulting from the freezing treatment rupture the membrane and ensure a greater leaching of the cell contents. The tissue was pulped in the manner described previously, but the tissue was washed three times with an excess of distilled water and oven dried at approximately 80°C.

The treatments consisted of extracting equal portions of the dried phloem with acetone and ethyl ether. This step was completed by mixing the dried phloem with an excess of the solvent in a food blender for two minutes. The pulped phloem was then exposed to a vacuum for thirty minutes. When solvent vapors were no longer evident, equal lots of acetone, ethyl ether and untreated phloem were placed in 12 small two-inch jars. To this substrate distilled water was added until each treatment had approximately similar moisture levels. At this time ten male Ips confusus were introduced into each jar and permitted to feed at room temperature (24 ± 4°C.) for 72 hours. All tests were performed in olfactometer number 2 with an air stream of 100 percent relative humidity.

The summary of data recorded in this test is given in Table 11. Due to the obvious differences between treatment and sector means, a factoral analysis of

variance test was not applied. Superficially, the results implicated a fat soluble substance(s) as responsible for enhancing the response elicited by the Ips confusus pheromone. The fact should be mentioned that a very slight odor was detected after the treatments were moistened with distilled water. The presence of a solvent residue immediately raises the possibility of inhibited feeding and, conversely, reduced pheromone production. This contingency is even more prevalent when the mean number of beetles per observation (Table 11) are compared with the mean number of beetles in Tables 6 and 7. It immediately becomes apparent that the values of 1.3 and 1.4, for acetone and ethyl ether respectively, could be a result of a humidity preference.

The textures of the homogenized phloem in both solvent treatments was quite different as compared to the control. The acetone treatment resulted in a cottony and pale appearing pulp, while the ethyl ether extract reduced the phloem to a very fine texture which approached a powder when dried. In addition, the pulp in the latter treatment had a distinct red color. The sector effect was again demonstrated, although a significantly larger number of beetles responded to sector 4 (i.e., each treatment appears four times in the arena circle) than sector 1. This disparity from the previous data indicated that the sector effect is probably a random

Table 11. Summary of the comparative response by female Ips confusus elicited by males feeding in homogenized phloem extracted with two different organic solvents

Main factor	Total number 1/ of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Treatments				
Control 2/	194	72.1	6.9	28
Acetone	37	13.6	1.3	28
Ethyl Ether	38	14.1	1.4	28
Sectors 3/				
1	41	15.1	2.0	21
2	46	17.1	2.2	21
3	73	27.1	3.5	21
4	109	40.5	5.2	21

1/ Test composed of seven replications with 100 beetles per replication

2/ No solvent extraction

3/ Arena divided into four sectors as a result of only three treatments

occurring event and of no particular significance in terms of olfactometer characteristics or behavioral traits of responding beetles.

The data from the antecedent experiment failed to conclusively implicate a fat-foluble material as the dietary factor essential for pheromone synthesis. As a result, an additional experiment was designed to examine

this consideration. The experimental design was also expanded in order to obtain additional information concerning the role of dietary sugar.

The experiment was implemented by removing an additional section of phloem from the same tree used in the preceding experiment. The procedure for pulping the phloem was identical to the manner described previously. The pulp, after being homogenized in the food blender, was washed three times with an excess of distilled water. The ethyl ether was omitted in this test, primarily due to the hazards associated with its inflammability. A portion of the pulp was extracted two times with an excess of acetone while being stirred continuously with a variable speed power stirrer. The pulp was removed, subjected to a vacuum for thirty minutes and air dried until an odor was no longer detectable. Equal shares of dried phloem were placed in six small two-inch jars, three of which were moistened with distilled water, while the remaining jars were moistened with a 1 molar solution of glucose. The balance of the pulp which had not been extracted with acetone was placed in the remaining six jars. Distilled water was added to the dried phloem in three jars with the remaining three being moistened with the water used in the initial pulping procedure. To each of the 12 jars ten male Ips confusus were introduced and permitted to feed 72 hours.

The results of this experiment, summarized in Table 12, infer that acetone soluble compounds are not a dietary requisite for pheromone synthesis. These data do not support the conclusion that male Ips confusus are less effectual in pheromone synthesis when feeding on phloem extracted with water and acetone. The support for this statement becomes apparent immediately when treatments 1 and 3 are compared (Table 12). The mean number of beetles responding to each treatment are statistically inseparable, thereby lending substance to the theory that the nutritional requisite is water soluble. This conjecture is sustained by treatment 4 which implicates glucose as an essential dietary factor. This treatment yielded an effect which was significantly better at the .5% level (Appendix VIII) than the effect from treatment 2 (Control).

The sector effect is once again observed (Appendix VIII). As discussed earlier, however, this effect is considered to be a random occurring characteristic. The interaction between replications and sectors was significant due to the high level of response to sector 2 on the third replication. The biological significance of this interaction appears slight. The highly significant (.5%, Appendix VIII) differences between treatments and sectors occurred as a result of the response to treatment 2 in sector 3 and conversely is of little apparent consequence.

Table 12.

Summary of the comparative response by female Ips confusus elicited by males feeding in homogenized phloem extracted with acetone and distilled water and supplemented with three types of solutions, e.g., 1 molar glucose, water used in homogenizing process, and distilled water

Main factor	Total number of beetles responding[1]	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Treatment				
1-Water extraction[2] + water supplement	96	21.4	4	24
2-Water extraction[3] + water supplement	89	19.8	3.7	24
3-Acetone extraction + water supplement	110	24.5	4.6	24
4-Acetone extraction + glucose supplement	154	34.3	6.4	24
Sector				
1	140	31.2	4.4	32
2	187	41.6	5.8	32
3	122	27.2	3.8	32

[1] Test composed of eight replications with 100 beetles per replication.

[2] Phloem extracted three times after pulping with an excess of distilled water.

[3] Extent of extracting was during pulping process; same water added back to pulped phloem.

The findings of this latter study and the preceding studies on carbohydrates support the conclusion that glucose is effectual as a dietary supplement in enhancing Ips confusus' pheromone synthesis, while acetone soluble components did not appear to be essential. In reference to the validity of these conclusions, it is essential to mention once again the implications of the statistical procedure followed during the course of these studies. The F value was used as the criterion for separating treatments; however, if replications are considered as a random factor then the statistical design necessitates the rejection of the error term as a constant in computing the F value. Instead, the denominator for each factor is determined by the method of expected mean squares (119, p.297). Accordingly, nearly all treatment effects would be insignificant and carbohydrates would then be considered ineffectual as a dietary supplement.

Olfactory Studies In Vivo of Dietary Carbohydrates

In order to re-enforce or refute the conclusions that dietary carbohydrates enhance the synthesis of the Ips confusus attractive principle, a series of olfactory tests were made on recently felled trees. The logic germane to this approach arises from the data of earlier experiments and data presented elsewhere.

Studies in vitro on dietary carbohydrates were

conclusive in suggesting certain sugars as effectual in intensifying the apparent level of Ips confusus' pheromone. At most, these studies failed to take into account such factors as sugar concentrations, combination of sugars (i.e., that a greater pheromone level may result from a synergistic affect), alteration of the natural medium and its influence on gustatory stimuli and other factors which undoubtedly affect the behavior of male Ips confusus and conversely, pheromone synthesis. To minimize these possible effects, a group of trees were selected for olfactory tests which varied in their respective osmotic values.

This approach of using intact trees with various osmotic pressures seemed consistent with the data of Gail (47) and Lewis and Tuttle (75). Observations on the freezing point depression of expressed sap from current years' needles of ponderosa pine prompted Gail to conclude that an increase in o.p. from July through November was the result of an accompanying increase in carbohydrates. After studying the expressed sap in needles of several coniferous and deciduous trees, Lewis and Tuttle decided that variations in the sugar content were correlated very closely with variations in the o.p., and it was concluded that non-electrolytes were principally responsible for the variation in osmotic values. These findings support the contention that trees with a comparatively low

osmotic value (7.5-8.5 atm.) are deficient in phloem sugar as compared to trees with a comparatively high osmotic value (11.5-14 atm.).

To initiate these field studies three types of trees were selected for the olfactory tests. The selection was based on their respective osmotic value. Two trees were characterized by their high and low o.p. The third tree was infected by Western gall rust (Cronartium harknessii (Moore)(Meinecke). This fungus attacks pines such as Jeffrey, ponderosa, lodgepole, and digger (14, p.196). The globose galls, formed by the aecia stage are effectual, after extensive development, in blockage of the descending sap stream. The infected tree contained a canker which nearly girdled the phloem except for a small bridge of uninfected tissue. This biological girdle was apparently responsible for the comparative high concentration of sugars above the canker and the comparatively lower concentration below the site of infection. The various attributes of the host material are given in Table 13. A total of three small billets, approximately 17 inches long were taken at breast height from trees 1 and 2. A similar number of billets were procured from the canker tree immediately above and below the site of infection which was nearly ten feet above the root collar. The billets were infested with 35 male Ips confusus and allowed to feed at room temperature for 72 hours.

Table 13. Characterization of ponderosa pine used in field olfactory studies commencing June 14, 1963

Tree	o.p.[1] (atm.)	o.e.p. (p.s.i.)	Phloem thickness[2] (thousandths of an inch)	Height (feet)	Age (years)	d.b.h. (inches)
1	13.0	170	.068	63	42	7
2	8.6	0	.037	55	39	6
3a[3]	12.8	128	.175	78	78	13
3b[4]	9.5	132	.079	78	78	13

[1] Average of seven readings

[2] Average of eight readings

[3] Based on readings taken approximately six inches above infection

[4] Based on readings taken approximately six inches below infection

The first billet removed distal to the canker was placed in a field olfactometer and situated in position one on the five meter circle. This position has an easterly inclination. The treatment assignments in the remaining three positions of sector 1 were accomplished in the following manner. Billets from the low o.p. tree, immediately below the canker, and the high o.p. tree were placed in positions two, three and four respectively. The treatment sequence was similar in the following two sectors. The field population responding to each of the 12 olfactometers was recorded every two hours starting at 0800 hours and terminating at 2000 hours. At 0800

hours for the following 11 days, each treatment was rotated one position in a clockwise direction. This procedure reduced the chance of biasing one particular sector with any given treatment.

Additional data were obtained by suspending the remaining portions of trees 1 and 2 in a horizontal aspect nearly four feet above the ground. The ends of each tree were supported by two steel fence posts, driven at an angle to each other of approximately 45 degrees. Six-foot sections were removed on each side of the canker and supported on steel "saw horses." To each tree eight male Ips confusus were introduced, and the ensuing attacks were marked and recorded at 1000 hours and 1500 hours for the following 11 days.

A factorial analysis of variance test (Appendix IX) reveals that the billets from above the canker were highly effective in trapping responding beetles of the field population of Ips confusus. A comparison of data given in Table 14 indicates that the descending order of treatments based on total numbers of captured beetles would be trees 3a, 1, 3b and 2. This order is of particular interest when the o.p. values of each type are compared with an unpaired "t" test. As apparent from Table 13, a statistical difference is not present between the osmotic values of the high pressure tree (Tree 1) and the area six inches above the canker infection (Tree 3a).

The comparison of the osmotic values between the area above and below the canker reveals a highly significant difference ($t = 15.75$, $t_{0.01}$ with 12 D.F. = 3.05). A similar comparison between trees 1 and 2 show an even greater significant difference ($t = 21.92$, $t_{0.01}$ with 12 D.F. = 3.05). When trees 1 and 3b are compared, a nearly equal significant dissimilitude ($t = 19.79$, $t_{0.01}$ with 8 D.F. = 3.05) is found. Therefore, it becomes apparent that a descending list of osmotic values can be constructed which parallels the list based on the total number of beetles per observation in Table 14.

The high ratio for days in Appendix IX is a result of the low number of beetles captured on the sixth and seventh days of the experiment. This appears to be due to low temperatures, for the average maximum-minimum of the preceding five days were 94.2 and 63.0°F. respectively. On the sixth and seventh days the same means were 74.5 and 40.5°F. During the next three days the mean daily temperature was down nearly ten degrees and was accompanied with heavy clouds and intermittent showers. Consequently, there is a period of three days between the seventh and eighth day in Table 14 in which no beetle flights were recorded. The 2601 Ips confusus responding on the ninth day probably are a result of an increase in flight amplitude rather than a change in the pheromone level.

Although not presented in Table 14, a bimodal diurnal flight pattern was observed in this experiment, similar to the report by Vité and Gara (130). The main variation from their data were the times of maximal flights in the a.m. and p.m. They recorded peaks on 0900 hours and 1700 hours whereas the maximal flights occurred during this study between 1000 and 1200 hours, and 1600 and 1800 hours.

The presence of a sector effect is evident from Table 14 and Appendix IX. Sector 3 was apparently preferred to sectors 1 and 2. This observation is at odds with the subsequent field olfactory tests. In these tests, almost without exception, the olfactometers in sector 1 were capable of trapping a greater portion of the responding beetles than the remaining two sectors. This appeared to be a result of the predominant southwest wind. Gara (48) demonstrated conclusively that Ips confusus fly with the wind, at which time they orient and fly upwind upon encountering the attractant principle. In that sector 1 has a southeasterly, which occasionally varies to a southwesterly attitude, it is not surprising that treatments within sector 1 captured the greater portion of the responding beetles. The variant from this explanation, as seen with the data in Table 14, could possibly be a result of a modification in wind direction. In mid and late summer the wind is, unquestionably, from

Table 14.

Summary of the comparative response to field olfactometers baited with billets of four physiological types, e.g., from trees with high o.p. and low o.p. and the portions above and below the site of infection caused by Cronartium harknessii

Main factor	Total number of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Days				
1	773	5.8	10.7	72
2	1648	12.5	22.9	72
3	1708	12.9	23.7	72
4	1277	9.7	17.7	72
5	1036	7.8	14.4	72
6	535	4.0	7.4	72
7	513	3.9	7.3	72
8	1906	14.4	26.5	72
9	2601	19.7	36.1	72
10	1129	8.5	15.7	72
11	103	.8	1.4	72
Treatment				
Above canker	5403	40.8	27.3	198
Below canker	2613	19.7	13.2	198
High o.p.	3881	29.3	19.6	198
Low o.p.	1332	10.1	6.7	198
Sector				
1	4283	32.4	16.2	264
2	3604	27.4	13.7	264
3	5342	40.4	20.2	264

the southwest; however, it is tenable that a different wind pattern was prevalent during the duration of this test (June 14, 1963 - June 26, 1963).

The interaction, significant at the .05 percent level, (Appendix IX) between days and time appears to be the result of an unseasonably low temperature (approximately 59°F) on the seventh day between 0800 hours and 1000 hours. The interaction between days and sectors which appeared in the statistical analysis was a consequence of the comparatively low numbers of beetles captured in sector 2 on the sixth day of the experiment. Any explanation, understandably, would be based on conjecture, due to the impracticability of measuring all factors which may influence Ips confusus' response pattern. However, it may be worthy of noting that a change of weather was forthcoming and a distince shift in wind patterns was detected.

The data of the preceding experiment sustain the contention that variances in host physiology, as reflected by osmotic values of expressed phloem sap, are manifested in levels of pheromone synthesis. This variance in o.p. is apparently a result of quantitative changes in soluble carbohydrates (47 and 75). When field olfactometers are baited with billets of the various physiological types, a correlation can be demonstrated between the numbers of responding beetles and the

quantitative aspects of the carbohydrates of each billet.

When the remaining portions of the trees, used in the previous experiment, were subjected to the field population of Ips confusus the attack pattern seems to be related to data obtained with the field olfactometers. During the 11-day period of this study, 616 (13.9 per square foot) Ips confusus males initiated attacks on tree 1 (Table 13) while only 210 (7.7 per square foot) were found on tree 2 (Table 13). As a result of a mistake in judgment, the logs from the canker tree were placed in an unshaded area and consequently very few attacks occurred.

Based on only two trees, it seems tenable to suggest that the high number of attacks per square foot on tree 1 was a result of the comparatively greater level of initial pheromone synthesis. This conjecture finds support when the daily attack records are examined. Three days after the introduction of the eight males, tree 1 had sustained an accumulative attack total of 150, whereas tree 2 had sustained 41 attacks for the comparable period.

The differences in osmotic values between the area above and below the canker was assumed to be the result of osmotically active material accumulating distal to the site of infection. It seemed reasonable to assume that ponderosa pine infected with western gall rust may show

variations in the values described for tree 3a and 3b (Table 13), especially when the fungus showed different degrees of phloem permation. Therefore, an additional pine infected with the fungus was selected for further olfactory studies.

This tree was comparable in many ways to the fungus infected tree in the preceding experiment. Characterization of this pine included the following attributes: o.p. above canker - 13.0 atm.; o.p. below canker - 11.6 atm.; o.e.p. above canker - 150 p.s.i.; o.e.p. below canker - 157 p.s.i.; phloem thickness above canker - .081 inches; phloem thickness below canker - .055 inches; height - 81 feet; age 70 years; d.b.h. - 12 inches. The same experimental procedure was followed as described for the preceding experiment. The tree was felled and four 15-inch billets were removed immediately above and below the canker. Each billet was infested with 35 male Ips confusus and permitted to feed for 72 hours. The arrangement of the olfactometers varied from the initial test. Positions 1, 6, 7 and 12 were eliminated. The first billet distal to the infection site was placed in position 2, position 3 contained the first billet proximal to the infection site. The olfactometer assignments followed in a similar fashion in the remaining three sectors. As before, olfactometers were checked every two hours from 0800 hours to 2000 hours. The experiment was terminated

after 48 hours. At 0800 hours on the second day, olfactometers in positions 2 and 3 were exchanged with positions 4 and 5, and similarly 8 and 9 with 10 and 11.

The t test on the osmotic values of the areas six inches above and below the infection perimeter indicated that differences are significant at the one percent level ($t = 3.94$, $t_{0.01}$, with 12 D.F. = 3.055). However, the fact is obvious that very little preference was manifested by the field population for either of the treatments (Table 15). The magnitude of this difference was not sufficient to indicate that it was not the result of chance (Appendix X).

The highly significant difference (.5 percent level, Appendix X) between 1400 hours and 2000 hours is simply a reflection of temperature. The maximal periods of flight for Ips confusus occur during the latter part of the a.m. and mid portions of the p.m. It is interesting to note that olfactometers in sector 2 were apparently more efficient in trapping responding beetles than the remaining six olfactometers. The sector effect is at variance with the results presented in Table 14 and Appendix IX. In the preceding experiment a significantly greater number of beetles responded to olfactometers within sector 3. The results of this test implicate sector 2 as the area preferred by the majority of responding beetles. The fact should be pointed out that the

Table 15.

Summary of the comparative response to field olfactometers baited with billets removed from the distal and proximal perimeters of a canker caused by Cronartium harknessii

Main factor	Total number of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Time				
1000	612	12.5	38.2	16
1200	765	15.6	47.8	16
1400	397	8.1	24.8	16
1600	400	8.2	25.0	16
1800	1081	22.1	67.6	16
2000	1651	33.6	103.2	16
Treatment				
Above canker	2272	46.3	47.3	48
Below canker	2634	53.7	54.8	48
Sector				
1	1355	27.6	56.4	24
2	2119	43.2	88.3	24
3	889	18.1	37.0	24
4	543	11.1	26.6	24

preceding test was composed of 12 olfactometers divided into three sectors; the latter test utilized eight olfactometers divided into four sectors. Therefore, discrete comparisons of sectors are difficult but the causal agent in both experiments is apparently the result of the prevailing winds.

The first study involving a ponderosa pine infected with western gall rust indicated that the area distal to the infection is characterized by an o.p. higher than a similar area proximal to the infection. In turn, these differences were manifested by the increased response of Ips confusus to olfactometers baited with billets distal to the canker. The second study failed to corroborate these findings in toto. These latter data suggested that osmotic values are significantly higher in the area above the infection; however, a correlation could not be established between this area and an increase in pheromone production. The difficulty in establishing the limits of mycelial permeation and conversely, the degree of phloem severance, prompted a series of studies in which trees were mechanically girdled.

The physiological effect of severing the descending conducting elements of most plants is well documented. Kramer and Kozlowski (71, p.522), in considering the basic aspects, stated, "The removal of inner bark cuts the active phloem and blocks downward translocation of

carbohydrates and growth substances from the crown. Therefore, carbohydrate accumulation just above the girdle results in abnormally thick growth in that area." After girdling yellow birch (Betula lutea Michx.), beech (Fagus grandifolia Ehrh.) and sugar maple (Acer saccharum Marsh), a marked quantitative change can be detected in phloem carbohydrates above and below the wound (3). Beech was peculiar in that a higher carbohydrate concentration was found proximal to the wound during the first year; however, on the subsequent year this pattern was reversed and the higher concentration was found distal to the wound. The remaining two species were typical in that large amounts of sugars accumulated above the girdle. Studies on the translocation patterns of C^{14} in Pinus contorta Dougl., infected with dwarf mistletoe (Arcenthobium americanum Nutt.) established that, in addition to the isotope, photosynthates accumulate distal to the infection (105). McDowell (92), while working on ponderosa pine infected with dwarf mistletoe (A. campylopodum Engelm f. campylopodum), detected an increase in carbohydrates in the area of infection. Shaw (115) was successful in demonstrating that carbohydrates accumulate at the wound site in leaves of sunflower and certain cerals. Experiments with Cryphalus piceae on branches of silver fir (Abies alba) established that osmotic values were normal in the area proximal to the

point of attack but abnormally high beyond the point of attack (22).

In order to examine further the effect of dietary carbohydrates on the magnitude of olfactory stimuli elicited by male Ips confusus, a group of trees, girdled for varying periods of time, were felled and cut into 15-inch billets above and below the wound. A total of 35 males were forced into each billet and allowed to feed for 72 hours. This group of trees included one tree which had been girdled for six years, two trees girdled for one year and two trees girdled approximately 30 days prior to the test. Osmotic values for the areas proximal and distal to the wound were obtained from the one- and six-year girdled trees. The osmotic values in the remaining two trees were obtained before the trees were girdled and again at the end of the 30-day period. Characterization of the trees is given in Table 16.

The results of the olfactory studies shown in Table 17 and Appendix XI represent data recorded in the latter part of July, 1963. Table 18 and Appendix XII set forth the data accumulated during the first two days of September, 1963. A total of three 18-inch billets were taken from both sides of the girdle in tree 1, whereas four 18-inch billets were removed from the same areas respectively in tree 2. This accounts for the fact that, during the olfactory study of tree 1, the arena was

Table 16. Characterization of ponderosa pine girdled for one month, one year, and five years

Tree	o.p.[1] (atm.)	o.e.p. (p.s.i.)	Phloem thickness[2] (thousandths of an inch)	Height (feet)	Age (years)	d.b.h. (inches)
1 month						
1a[3]	13.2	-	.100	51	50	7
1b[4]	12.3	142	.086	51	50	7
2a	10.9	-	-	83	95	11
2b	10.4	132	.037	83	95	11
1 year						
3a	12.5	132	.091	51	41	8
3b	7.9	148	.081	51	41	8
4a	15.1	130	.178	53	36	9
4b	10.6	132	.116	53	36	9
6 years						
5a	15.3	132	.117	96	76	13
5b	12.7	135	.082	96	76	13

[1] Average of seven readings

[2] Average of eight readings

[3] Based on readings taken approximately six inches above wound

[4] Based on readings taken approximately six inches below wound

divided into three sectors, each of which contained two billets from different areas. In the care of tree 2, the billets were assigned in such a manner that the arena was divided into four sectors. During the flight period, i.e., from 0800 hours to 2000 hours, numbers of Ips confusus trapped in each olfactometer were recorded at each two-hour interval.

The fact is obvious (Tables 17 and 18, Appendixes XI and XII) that a greater number of Ips confusus responded to billets proximal to the girdle. Although the difference between the two areas was not as significant statistically in tree 2 (.05% level, Appendix XII) as tree 1 (.005% level, Appendix XI), it should be pointed out that in both trees the percent of total beetles responding to each area were nearly identical. In addition, an examination of the osmotic values of the areas indicates that changes which occurred in the area distal to the girdle were of the same magnitude in both trees. A t test of the dissimilitudes in osmotice values indicated a difference in tree 1 significant at the one percent level ($t = 10.88$, $t_{0.01}$, with 12 D.F. = 3.055); in tree 2 the difference was also significant at the one percent level ($t = 12.91$, $t_{0.01}$, with 12 D.F. = 3.055). Therefore, the differences between the billets in tree 2 are considered to be an effect of the girdle even though the statement was made in the discussion on statistical

analysis that differences significant at the five percent level would constitute a basis for accepting the null hypothesis. Prior to girdling the trees, osmotic values of each area were determined and significant differences could not be detected, i.e., difference of .8 atm. between areas in tree 1 and difference of 1 atm. in tree 2.

The data on the remaining main factors, e.g., time and sectors, re-enforce the former observations. A bi-modal flight pattern was again demonstrated as described by Vité and Gara (129). The response maximums for tree 1 occurred somewhat earlier than the diurnal maximums for tree 2, but variations in diurnal temperature fluctuations appear to be responsible. The effect of the prevailing winds was exhibited by the preference responding beetles showed for a particular section of the concrete arena. Although there is some variation between studies, a factoral analysis of variance indicated, in both studies, a difference significant at the .5 percent level.

It seemed reasonable to expect a correlation between osomotic values and the length of time that the descending sap stream was interrupted. The correlation should then be paralleled by an increase in response to field olfactometers baited with billets from the different portions of the girdled tree. To extend this hypothesis, two trees which had been girdled the preceding summer

Table 17. Summary of the comparative response to field olfactometers baited with billets taken distal and proximal to a one-month-old girdle (tree 1a and 1b)

Main factor	Total number of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Time				
1000	475	12.4	29.7	16
1200	403	10.6	25.2	16
1400	719	18.8	44.9	16
1600	818	21.4	51.1	16
2000	1401	36.7	87.5	16
Treatment				
Above girdle	2302	60.3	57.6	40
Below girdle	1513	39.6	37.8	40
Sector				
1	984	25.8	49.2	20
2	1776	46.6	88.8	20
3	574	15.0	28.7	20
4	481	12.6	24.1	20

Table 18. Summary of the comparative response to field olfactometers baited with billets taken distal and proximal to a one-month-old girdle (tree 2a and 2b)

Main factor	Total number of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Time				
1000	134	8.9	11.2	12
1200	209	13.9	17.4	12
1400	143	9.5	11.9	12
1600	195	13.0	16.2	12
1800	463	30.8	45.4	12
2000	361	24.0	30.1	12
Treatment				
Above girdle	907	60.2	25.2	36
Below girdle	598	39.7	16.6	36
Sector				
1	642	42.6	26.8	24
2	302	20.1	12.6	24
3	561	37.7	23.4	24

were selected for study. The experimental design was identical to the procedure described in the prior two experiments. The first olfactory study was initiated on the 31st of July, 1963; the second on the 8th of August, 1963. Both tests had a duration of 34 hours. A brief description of the ponderosa pine used in this study are given in Table 16.

As a result of the girdle, considerable differences in osmotic values were apparent between the comparable portions of each tree. A t test applied to the osmotic data shown in Table 16 demonstrated a highly significant intraspecific difference within each tree, e.g., tree 3, $t = 15.17$, $t_{0.01}$ with 12 D.F. = 3.055; tree 4, $t = 12.55$, $t_{0.01}$ with 12 D.F. = 3.055. A similar test applied to the phloem measurement data for tree 4 indicated that the increase in thickness was significant at the one percent level ($t = 5.5$, $t_{0.01}$ with 14 D.F. = 2.977); an increase in phloem thickness had occurred in tree 3; however, the difference was not significant at the five percent level ($t = 1.24$, $t_{0.05}$ with 14 D.F. = 2.145).

A factorial analysis of variance (Appendixes XIII and XIV) on the number of responding Ips confusus sustains the hypothesis that an increase in host o.p. is paralleled by an increase in pheromone synthesis. A factorial analysis of variance (Appendixes XIII and XIV) of data summarized in Tables 19 and 20 support the

conclusion set forth in the preceding set of experiments. A direct correlation appears to exist between o.p. and suitability of the host as a substrate for pheromone snythesis. The data presented in these two studies conclusively demonstrated that an increase in host o.p. directly affects the apparent level of pheromone synthesis. Differences between the distal and proximal billets of trees 3 and 4 were extremely significant (i.e., tree 3, $F = 66.30$ $F_{0.005}$ with 1 and 15 D.F. = 10.798; tree 4, $F = 142.99$ $F_{0.005}$ with 1 and 15 D.F. = 10.798.

The time and sector effects have been considered in the previous olfactory studies and these results do not require further explanation. The bimodal flight curve is again prevalent with the peaks occurring in the late a.m. and mid p.m. The sector effect, for the most part, parallels the earlier results. Treatments assigned to sectors 1 and 2 are more effective in trapping beetles than the remaining sectors.

Table 19. Summary of the comparative response to field olfactometers baited with billets taken distal and proximal to a one-year-old girdle (tree 3a and 3b).

Main factor	Total number of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Time				
1000	500	10.2	31.2	16
1200	731	15.0	45.7	16
1400	409	8.4	25.6	16
1600	438	9.0	27.4	16
1800	1078	22.1	67.4	16
2000	1726	35.4	107.9	16
Treatment				
Above girdle	3288	67.3	68.5	48
Below girdle	1594	32.6	32.6	48
Sector				
1	1558	31.9	64.9	24
2	1793	36.7	74.7	24
3	774	15.8	32.6	24
4	757	15.5	31.5	24

Table 20. Summary of the comparative response to field olfactometers baited with billets taken distal and proximal to a one-year-old girdle (tree 4a and 4b)

Main factor	Total number of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Time				
1000	273	11.8	17.1	16
1200	404	17.4	25.2	16
1400	273	11.8	17.1	16
1600	335	14.4	20.9	16
1800	752	32.4	47.0	16
2000	284	12.2	17.8	16
Treatment				
Above girdle	1823	78.5	38.0	48
Below girdle	498	21.4	10.4	48
Sector				
1	625	26.9	26.0	24
2	617	26.6	25.7	24
3	834	35.9	34.8	24
4	245	10.6	10.2	24

The results of the preceding experiments indicate that intraspecific differences as measured by numbers of responding Ips confusus are greater in trees girdles for one year than trees girdled for one month. To extend this observation, a single tree girdled on April 18, 1957 was selected for olfactory study. Characterization of this tree is given in Table 16.

For the most part the experimental design was identical to the manner described previously. The tree was felled on July 24, 1963 and two billets were removed from each side of the girdle. Due to the large diameter, each billet was split in half. Arena positions 1 and 7 were eliminated from the test; billets distal to the wound were assigned to positions 2, 4, 8 and 10, the billets proximal to the girdle were assigned to the remaining arena positions. The test was initiated on July 26 and terminated on July 27. Beetles responding to each olfactometer were collected at two-hour intervals commencing at 0800 hours and ending at 2000 hours.

Contrary to the earlier results, a significant increase in response to olfactometers baited with billets situated above the girdle could not be demonstrated (Appendix XV). This fact is of particular interest, especially when the comparable o.p. values are examined (Table 16). The application of a t test indicated that the osmotic value in the upper billets (15.3 atm.) was

significantly greater at the one percent level (t = 11.28, $t_{0.01}$ with 12 D.F. = 3.055) than the o.p. in the lower billets (12.7 atm.). It is of interest to note that the latter osmotic value is exceptionally high for a tree whose root system has been apparently deprived of photosynthates and other essential organic material for a period in excess of six years.

The fact that both areas were characterized by a comparatively high o.p. plus the seeming thriftiness of the tree indicated that the root system was being supplied with carbohydrates from neighboring trees. Although this conclusion is based on conjecture, Kramer and Kozlowski (71, p.213) lend support to this theory by their statement, "A group of trees may be connected so effectively through root grafts that if all but one tree are cut, the root systems will survive on carbohydrates supplied by the remaining tree." Provided this tree's root system was interconnected to adjacent trees, an explanation could then be offered for these anomalous results.

The summation of data in Table 21 illustrates the effect of the remaining main factors. A plausible explanation cannot be proposed for the large increase in response to treatments during the second day of the experiment. The temperature maximums for the two days were 33 and 34^{o}C. respectively. The highly significant

Table 21. Summary of the comparative response to field olfactometers baited with billets taken distal and proximal to a six-year-old girdle (tree 5a and 5b)

Main factor	Total number of beetles responding	Percent of total of (approximately)	Mean number of beetles per observation	Total number of observations per factor
Days				
1	1308	24.4	27.2	48
2	3834	74.6	79.9	48
Time				
1000	343	6.7	21.4	16
1200	527	10.2	32.9	16
1400	449	8.7	28.1	16
1600	514	10.0	32.1	16
1800	1048	20.4	65.0	16
2000	2261	44.0	141.3	16
Treatment				
Above girdle	2867	55.8	59.7	48
Below girdle	2275	44.2	47.4	48
Sector				
1	1610	31.3	67.1	24
2	2702	52.5	112.6	24
3	562	10.9	23.4	24
4	268	5.2	11.2	24

effect of time (Appendix XV) was a result of the differential in response during the first and last two hours of each day. The sector effect has been considered in detail and is a consequence of the prevailing south-west winds. The factorial analysis of variance indicated interactions between day and time, and time and sectors. These interactions were significant at the five percent level and consequently were assumed to equal zero.

Laboratory studies. The preceding olfactory studies demonstrated the correlation between the osmotic values and the intensity of olfactory stimuli. This correlation was determined by comparing the relative numbers of Ips confusus responding to field olfactometers baited with various types of host material. The question remained, however, if similar relationships could be established under laboratory conditions.

This question was examined by selecting trees with expressed phloem sap of two basic physiological types, e.g., high o.p., low o.p., and intraspecific differences in o.p. resulting from the girdling effect of Western gall rust. The 85 mm circular sampler (Figure 7) was used to obtain three discs from each of the four physiological types. A total of 15 male Ips confusus were forced into each disc. The beetles were confined within each disc by stapling a circular piece of window screen to the bark surface. The exposed xylem of each disc was

waxed to prevent excessive moisture loss, and then inverted in a paper funnel. The frass from each disc was collected at the termination of the first and second 72-hour period. The comparative attractiveness of the frass was tested in a laboratory olfactometer. Conditions were similar to those described for earlier laboratory studies. The frass tested at each arena position was standardized in weight, thereby reducing the variation in response which would arise through quantitative differences.

The results of this study set forth in Table 22 and Appendix XVI complement the initial field study on dietary carbohydrates. The frass from trees 2 and 3b, characterized by a comparatively low o.p., e.g., 8.8 atm. and 11.1 atm. respectively, elicited a significantly lower response than frass from trees 1 and 3a, e.g., 11.3 atm. and 13.2 atm. respectively. The differences in osmotic values between trees 1 and 2 are highly significant ($t = 12.18$, $t_{0.01}$ with 12 D.F. = 3.055). Similarly, a highly significant difference ($t = 10.36$, $t_{0.01}$ with 12 D.F. = 3.055) was detected between the comparable regions of the canker tree (3a and 3b). A consideration of average osmotic values suggests that intensity of response data (Figure 17) are not correlated to the absolute osmotic figure. This fact is perceptible when trees 1 and 3b and 1 and 3a are compared. In the

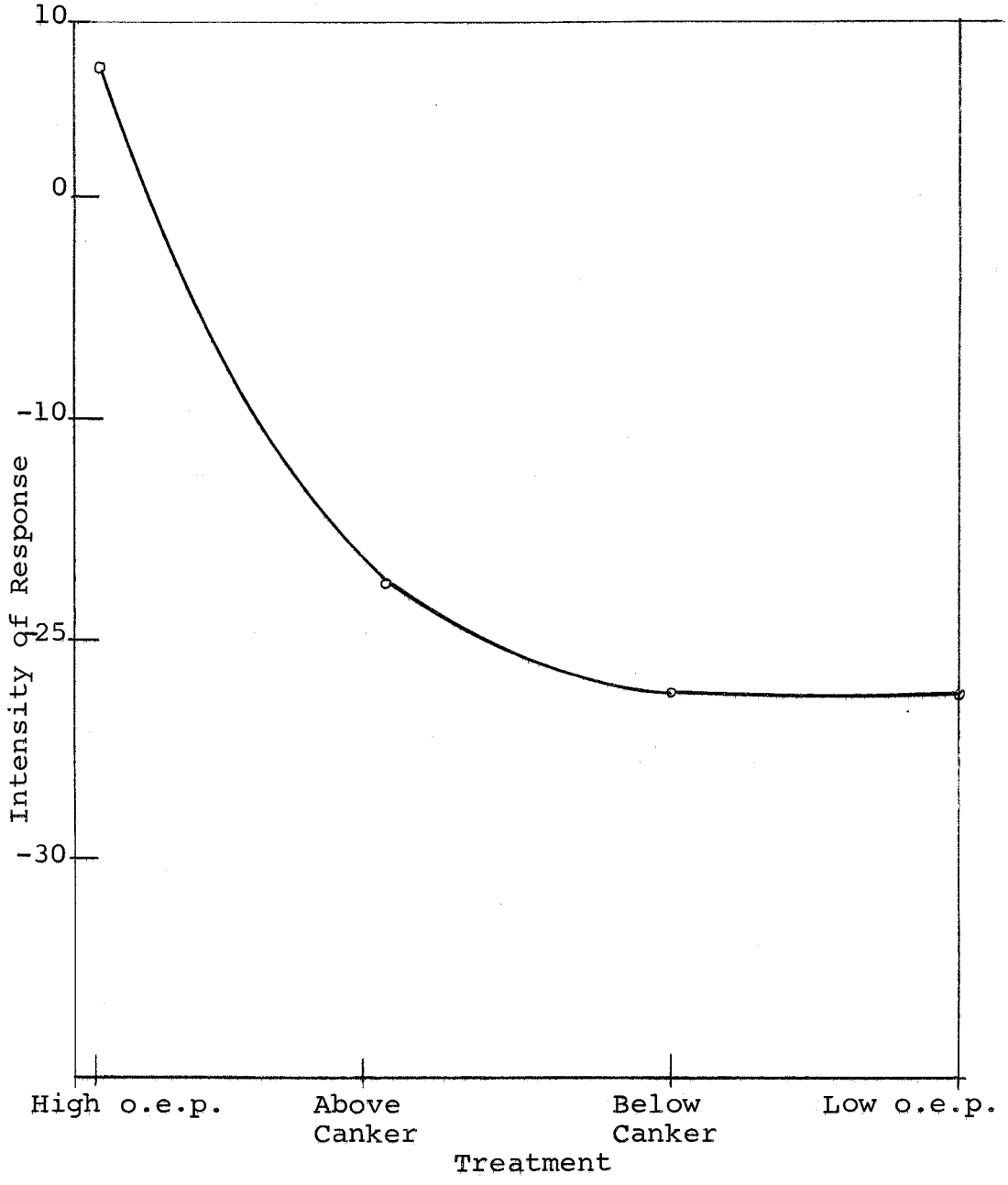

Figure 17. Intensity of response by female Ips confusus elicited by males feeding in discs from trees with high and low oleoresin exudation pressures and discs from the portions distal and proximal to a canker caused by Cronartium harknessii

Table 22.

Summary of the comparative response by female Ips confusus to frass produced by males feeding in 3-1/2-inch discs of four physiological types, e.g., trees with high o.p., low o.p., and above and below a stem canker caused by Cronartium harknessii

Main factor	Total number[1] of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Day				
3	449	56.6	6.2	72
6	344	43.4	4.8	72
Treatment[2]				
High o.p.	310	39.1	8.6	36
Low o.p.	136	17.2	3.8	36
Above canker	210	26.5	5.8	36
Below canker	137	17.3	3.8	36
Sector[2]				
1	378	47.7	7.9	48
2	255	32.2	5.3	48
3	160	20.2	3.3	48

[1] Test composed of five replications with 100 beetles per replication

[2] Represent totals from third and sixth day

former comparison, osmotic values are statistically inseparable, but dissimilitudes in response are highly significant at the .5 percent level. In the later comparison, tree 3a's mean osmotic value is significantly greater at the one percent ($t = 11.50$, $t_{0.01} = 3.055$) than the corresponding value in tree 1. However, in regard to the decision on rejecting all five percent values, a t test indicates that the difference between response means is zero ($t = 2.73$, $t_{0.01}$ with 22 D.F. = 2.831).

A defensible explanation for these anomalous results would be difficult to propose. The earlier conclusions regarding carbohydrates and their impact on feeding male Ips confusus appear correct but in view of above data it appears tenable to suggest that another material may also be essential for increased production of the attractant principle.

The factorial analysis of variance (Appendix XVI) test demonstrates, in addition to treatment effects, several main factor and main factor interactions. In view of the fact that a plateau in response is recorded three days after the initial infestation (130), it appears tenable to suggest that dissimilitudes between the first and second 72 hours are a result of the beetle per se. Variation in the ability to respond to field olfactometers has been demonstrated (48). As previously

discussed, the preference for a particular sector is random and of little consequence. The three sets of interactions (Appendix XVI) resulted from a comparatively high number of beetles responding to the first 72 hours' frass from tree 1; the replication sector interaction occurred as a consequence of the large numbers of females trapped in the first sector on the fourth replication; the disproportionately low numbers responding to tree 3b in the third sector accounted for the treatment sector interaction.

Olfactory Studies of Host Material Taken at Various Stem Heights

The seasonal attack pattern for *Ips confusus* is well documented (51 and 121). Beetles emerging from logging and clearing slash and storm breakage from the preceding winter are responsible for the main attack in mid summer and early fall. As a rule, the whole stem of young pole-size timber is attacked; however, tops of the larger d.b.h. classes also can sustain *Ips* infestations. Invariably this latter pattern of attack occurs in late summer and early fall.

In view of the data heretofore presented, it seemed reasonable to expect differences in response patterns elicited by males feeding in billets obtained from various heights of the stem. The premise for this

supposition rests upon the fact that an apparent correlation exists between osmotic values of expressed phloem sap and the intensity of olfactory stimuli. Past studies on certain trees have demonstrated a decline in osmotic values of phloem tissue as the distance from the crown increases. Merker (84), while working on Norway spruce (Picea abies), found that normal summer values were 12 atmospheres immediately below the crown and seven atmospheres at the roots. Zimmerman (147) reported a general decrease in sugar from the crown to the root collar of white ash (Fraxinus americana). Chararas (24), working independently, paralleled Merker's results and demonstrated that in Norway spruce the o.p. increased from five atmospheres at two meters to 16 atmospheres at 20 meters.

In order to test the above supposition, two trees were felled and billets removed at four different heights. Tree 1 (Table 23) was felled on July 25, 1963 and three billets taken from each interval of 0 - 4.5 feet, 10 - 14.5 feet, 20 - 24.5 feet and 30 - 34.5 feet were infested with 35 male Ips confusus. The experiment initiated July 28, 1963 followed the same experimental design as described for the earlier field olfactory studies. The five-meter concrete arena was used and the billets were assigned to olfactometers in the following manner. The terminal billet from the

fourth interval (30 - 34.5 ft.) was placed in position 1; the terminal billets from the remaining three intervals were placed in positions 2, 3 and 4 respectively. Similarly, the billets from the four intervals were assigned to the remaining nine olfactometers. The experiment had a duration of 60 hours and counts were made at intervals reported earlier. At the end of the 24-hour period each olfactometer was rotated one position to the right. An experiment with an identical design and purpose was initiated on August 12, 1963. Portrayal of the trees studied in these tests are presented in Table 23.

Table 23. Characterization of ponderosa pine used in field olfactory studies commencing July 25th and August 12th, 1963

Tree	o.p.[1] (atm.)	o.e.p. (p.s.i.)	Phloem thickness[2] (thousandths of an inch)	Height (feet)	Age (years)	d.b.h. (inches)
1	11.6	135	.049	51	57	8.2
2	12.3	111	.058	59	53	7

[1] Average of seven readings

[2] Average of eight readings

The results of these studies do not directly corroborate the findings of Merker (83), Zimmerman (146) and Chararas (24); however, there is a strong indication that a similar gradiation is present in ponderosa pine

as shown for Norway spruce and white ash. Support for this statement is visible from the results presented in table 24, Appendixes XVII and XVIII. It is apparent that a significantly greater number of beetles responded to olfactometers baited with billets from interval 4 than to those from interval 1. The numbers of beetles responding to intervals 2 and 3 preclude their separation. The evidence is indirect, but highly suggestive that o.p. gradients were responsible for response dissimilitudes. It appears germane to this set of studies to bring attention to the experimental design and total numbers of insects responding in each test.

The sector effect was minimized by rotating position assignments, thereby reducing the chance of biasing a particular treatment. Probably the most cogent element of these tests was the fact that 24,380 beetles were trapped during the six-day duration of both experiments. As a corollary, a tenable conclusion would implicate osmotic values and conversely complement the precedent set of studies on dietary carbohydrates.

For the sake of brevity, the summation data on time and sectors effects were omitted in Table 24. These factors exhibit similar effects as discussed in the earlier olfactory studies. The highly significant value for differences in means between the first and third day in the August 12th test does not appear to be a result

Table 24.

Summary of the comparative response to field olfactometers baited with billets taken at four different stem heights, e.g., 0-4.5, 10-14.5, 20-24.5 and 30-34.5 feet

Tree	Main factor	Total number of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
1	Days				
	1	3315	23.0	46.0	72
	2	4719	32.8	65.5	72
	3	6371	44.2	88.5	72
2	Days				
	1	1687	16.9	23.4	72
	2	2952	29.6	41.0	72
	3	5336	53.5	74.1	72
1	Height				
	1	2327	16.2	43.0	54
	2	3867	26.8	71.6	54
	3	3320	23.0	61.5	54
	4	4891	34.0	90.6	54
2	Height				
	1	1876	18.8	34.7	54
	2	2417	24.2	44.8	54
	3	2249	22.5	41.6	54
	4	3433	34.5	63.6	54

of temperature changes. An examination of the temperature records shows that maximum and minimum values for the 11th and 12th of August are in phase with values for the following two days. It must be concluded that this effect is due to fluctuations in beetle emergence.

Olfactory Study of Host Material Selected According to Phloem Thickness

The data presented in Table 4 support the contention that intraspecific differences in phloem thickness are related to the stem height. For the trees studied, it appears that an increase in phloem dimensions occurs until approximately midcrown, whereupon the trend is reversed and the phloem decreases in thickness. While investigating the brood mortality and survival of D. monticolae, Reid (106) found that the outerbark of western white pine decreases as the distance from the tree butt increases; no reference was made to inner living bark changes. When the data of Tables 4 and 24 are compared it becomes evident that the magnitude of response was greates to the olfactometers baited with billets from the stem portion with the thickest phloem.

To examine the possibility that dissimilitudes in response patterns may be correlated to variations in phloem thickness, a group of four trees was selected according to their phloem dimensions at breast height. It seemed essential to include trees which represented

the extreme in thickness and thinness as determined by the apparent norm in the B.T.I. Experimental Forest. To this end, it was necessary to sample approximately 40 trees. As a result, the trees selected were from different sites, age and dominant classes. Characterization of these trees is presented in Table 25.

Table 25. Characterization of ponderosa pine used in field olfactory studies commencing August 4, 1963

Tree	o.p.[1] (atm.)	o.e.p. (p.s.i.)	Phloem Thickness[2] (thousandths of an inch)	Height (feet)	Age (years)	d.b.h. (inches)
1	13.0	150	.146	45	51	8
2	15.0	93	.060	93	101	9.5
3	11.4	91	.034	32	57	4.8
4	10.7	80	.026	86	94	10.6

[1] Average of seven readings

[2] Average of eight readings

The experimental design and other basic attributes of this study are identical to several of the field studies described earlier. A single billet from each of the four trees was placed in 12 field olfactometers. Olfactometer placement on the various arena positions was accomplished in the following manner. An olfactometer containing a billet from tree 4 (Table 25) was

assigned to position 1 in sector 1. Billets from trees 3, 2 and 1 were assigned to positions 2, 3 and 4 respectively. The olfactometers containing billets from each of the four trees were placed in the remaining two sectors according to the preceding scheme. The duration of this particular test was 36 hours. Responding Ips confusus were collected at each two-hour interval from 0800 to 2000 hours.

The results which were anticipated from this study did not materialize. The point was reasoned that beetles feeding in phloem less than the average dorsal-ventral dimension of male Ips confusus would be forced to ingest greater quantities of bark. A dietary increase of nutritionally poor material may in turn affect the beetles' ability to synthesize the attractant principle. The dorsal-ventral dimension was determined, using the paper gauge micrometer, by measuring 100 responding male beetles. The mean value was determined to be .056 inches with a standard deviation of ± .0032 inches. For additional information, a similar number of responding females were measured and their average value was .055 inches with a standard deviation of .0028 inches.

It is apparent that the results obtained in the first 36 hours (Table 26) do not sustain the above theory. Tree 3, with an average phloem thickness nearly .020 of an inch thinner than the male beetle, was capable of

eliciting the highest response. The factorial analysis of variance (Appendix XIX) indicates that a difference, significant at .5 percent, was evident between mean numbers of beetles responding to billets from trees 3 and 4. It is of interest to note the o.p. of trees 3 and 4, cf. 11.4 atm. and 10.7 atm. respectively.

When the totals for each two-hour interval during the first 36 hours are used for estimating the dissimilitudes between billets from trees 1, 2 and 3, a t test indicated that the differences between means are zero ($t = 1.41$, $t_{0.05}$ with 10 D.F. = 2.228). A close examination of each olfactometer revealed that the ends of the billets from trees 1 and 2 were heavily coated with exuded oleoresin. Preliminary observations had indicated that Ips confusus were intolerant of oleoresin and the presence of this material showed marked interference in olfactory studies. The point was reasoned that olfactometers containing billets from trees 1 and 2 may be superior in eliciting olfactory response but the resin was masking the pheromone.

The data recorded in the following 36 hours are a result of this consideration. Slabs approximately three inches wide were removed from each billet. Care was exercised to avoid injuring the males contained in each billet. The exposed xylem was washed with ethyl alcohol, thereby removing the majority of the surface oleoresin.

Table 26.

Summary of the comparative response to field olfactometers baited with billets and slabs taken from trees with four different phloem dimensions, e.g., .146, .060, .034 and .026 inches

Main factor	Total number of beetles responding	Percent of total (approximately)	Mean number of beetles per observation	Total number of observations per factor
Treatments				
Billets	6513	55.8	45.2	144
Slabs	5155	44.2	35.8	144
Phloem[1/]				
.146	3326	28.5	46.2	72
.060	2804	24.0	38.9	72
.034	3665	31.4	50.9	72
.026	1873	16.0	26.0	72
Days				
1	2649	22.7	36.8	72
2	3864	33.11	53.7	72
3	2559	21.9	35.5	72
4	2596	22.2	36.0	72

[1/] Average of eight readings

The billets from the various trees were reassigned to the field olfactometers in a similar fashion as described earlier.

When the totals for each tree are compared (Table 26) it is apparent that the alcohol treatment had an effect although it can not be demonstrated statistically. The total magnitude of this effect, as determined by responding beetles, was nearly 1200. The factorial analysis of variance test (Appendix XIX) indicates that significantly (.5 percent level) more beetles responded in the first 36-hour period than in the second 36-hour period. This observation is not necessarily at odds with the explanation offered for trees 1, 2 and 3. Fluctuations in numbers of beetles responding from one day to the next was a common observation; consequently it becomes difficult to assess treatment effects in any other way than on a comparative basis.

The highly significant treatment effect is a result of the difference between trees 3 and 4. Equally significant were the differences observed for time and sector factors. These dissimilitudes are in accord with earlier observations and demand no further explanation. The day factor was evident as a result of differences in response patterns between the second and fourth days. For reasons that are not obvious, the largest flight was recorded on the second day. The interaction between

billets and treatments occurred as a consequence of the comparatively low response to olfactometers containing billets from tree 4 which had been slabbed and washed with alcohol. No supportable explanation can be offered for this particular observation.

All second and third interactions in Appendix XIX have been purposely omitted. Only those values which are essential for computing the F ratio are included. Presentation of all sum of squares and mean square values for a five-factor model would present an unwieldy table, particularly when it is so difficult to offer sound biological explanations for many of the first order interactions. F ratios are computed on the basis of the average mean squares shown in Tables 2 and 2a.

DISCUSSION

Clinical Indices

Rudinski (109), in his comprehensive coverage of Scolytidae literature, brought attention to the need for assessing the physiological condition of a tree, e.g., water balance. To this end Vité and co-workers (128, 131, 133, 134 and 144) developed the technique of measuring oleoresin exudation pressure. This procedure is a rapid and dependable method for portraying the water balance of a tree. However, Rudinsky (109) reflects upon the study of water balance *per se* and states, "Not the quantity but the state of water is important."

The present studies on clinical indices indicate that osmotic values of expressed phloem sap are a reliable indicator of the physiological profile of ponderosa pine. Nearly without exception, a close correlation existed between o.e.p. and o.p. Trees with an apparent chronic state of water stress manifest not only a reduced o.e.p. but also a low o.p. Conversely, trees in the reciprocal condition possessed a comparatively high o.e.p. and o.p. This statement must be tempered with the fact that a state of acute water stress is usually a transitory condition and correctable through adaptation of the root system and transpiration surface (128). Therefore, it is to be anticipated that a correlation between these

two indicators may diverge in trees in a transitory condition.

The element common to an osmotic system, regardless of its expression, e.g., o.e.p. and o.p., is osmotically active materials. Deprivation of this element in a system of living cells eventually results in the reduction of cell turgidity, heedless of the available water. The diminution of turgor, in reference to oleoresin exudation, results in a marked reduction of pressure (128). The principal osmotically active materials in expressed phloem sap are non-electrolytes, mainly photosynthates (75). Therefore, a tenable explanation for the correlation between these two indicators would point to photosynthates. In reference to the role of water in photosynthesis, Meyer and Anderson (87, p.356) state, "...a reduction in the water content of leaves usually results in a decrease in the rate of photosynthesis...." A reduction of photosynthates would then be one of the syndromic expressions of a tree under chronic water stress and would equate a depressed o.e.p. and the low osmotic value of expressed phloem sap.

Although it was possible to propose the osmotic value of expressed phloem sap as a clinical index, it is not assumed that the physical nature of this tissue, e.g., thickness, is as applicable an indicator. There appears, however, to be a fairly high frequency of low o.p. and

o.e.p. trees with thin phloem (less than .050 of an inch). The observed range for ponderosa pine in and about the B.T.I. Experimental Forest was .026 to .250 of an inch. The lack of an extensive survey precludes establishing cogent categories which would reflect upon a tree's physiological profile.

The quantitative expression of phloem moisture, as computed on the basis of relative turgidity (5), does not appear to be reliable in ponderosa pine. The difficulty inherent to this technique seems to be the high amount of leaching which occurs during the soaking process. The present observations indicated that the osmotic value of ponderosa pine sap has a latitude of 7.7 atm. to 15.3 atm. This range would ostensibly result in a value different than shown in Table 5.

Blue-Stain Fungi Studies

Based on the present findings, it is not possible to characterize the fungi-bark beetle association as an obligatory symbiosis. Past studies (132) on *Ceratocystis* species associated with *Dendroctonus brevicomis* demonstrated that beetles can successfully overcome ponderosa pine in the absence of fungi. The fact has been demonstrated that the level of pathogenicity in *Ceratocystis* species is of the degree that trees of poor physiological vigor, e.g., low o.e.p., will succumb to a

blue-stain fungi infection (132 and 81).

The association between Ips confusus and Ceratocystis ips is probably best described as a facultative symbiosis. The present studies and those of other workers (16, 72, 116 and 132) suggest that the fungus is adding colonization and brood development in no other way than through reducing moisture movement and oleoresin exudation in the outer sap wood. Ips confusus larvae have been unable to develop in trees with an o.e.p. greater than ten pounds per square inch (141).

Probably one of more important roles of the microorganism is to reduce the resin flow in the immediate vicinity of the incubating eggs. It would be expected that first instar larvae, immediately after eclosion, are extremely sensitive to exuding oleoresins. The embryogenesis of Ips confusus is of sufficient duration to permit spore germination and hyphal permeation into the xylem immediately adjacent to the egg niches, resulting in resin blockages. Therefore, the beetles aided by the fungus are capable of overcoming certain trees which would otherwise be resistant. As apparent from the number of trees which are resistant to bark beetle attack, o.e.p. can be of sufficient magnitude to daunt the combined efforts of both organisms.

Factors Influencing Host Colonization

Past studies (48, 141 and 143) have demonstrated that Ips confusus' ability to respond varies within broods and between populations. The later emerging members of a brood as well as beetles emerging later in the season have shown a noticeable reduction in their capacity to respond. The above workers concluded that recorded dissimilitudes in response ability are due to physiological conditions of the insects, implying that physiological categories can be erected for members of each brood and population.

The present studies indicated that additional dissimilitudes in response patterns can be related to changes in host physiology. The laboratory studies indicated that variations in phloem sugar markedly influence Ips confusus' ability to produce the attractant principle. Several of the carbohydrates tested showed activity but glucose was consistently more effectual. Enough variability in the data was observed to preclude an unqualified implication of glucose as the single most important dietary element.

Olfactory studies on intact and girdled trees support, although somewhat indirectly, the contention that phloem sugars are responsible for the observed differences. The influence of carbohydrates was demonstrated by forcing male beetles into billets with various

levels of phloem sugar.

Although variation in osmotic values of expressed phloem sap were easily correlated to response differences, the possibility still exists that additional phloem factors are paralleling the carbohydrate changes. As an example, thiamine and pantothenic acid are the major vitamins in the descending sap stream and may be essential dietary requisites for pheromone synthesis. These water soluble vitamins are necessary for root differentiation and therefore accumulate in the distal portion when a tree is girdled. It is of interest to note that several of the B-vitamins are an absolute necessity for growth and development in many of the insect studies (108, pp.370-371). In addition to vitamins, the parenchymous tissue and sieve elements of phloem contain numerous nitrogenous compounds, hormones, auxins and other types of organic materials.

After examining the present findings it appears possible to offer a plausible explanation for the peculiar late summer attack pattern of Ips confusus. In that Ips can not overcome trees with an o.e.p. in excess of ten p.s.i. (141), it is not surprising that successful attacks would occur in the upper crown. The greatest water deficit can be recorded in this region. In the upper corwn the ratio of sapwood surface (tangential plane) to sap volume is large, particularly in comparison

to the butt portion. The manifestation of o.e.p. is dependent upon an adequate water supply (128), subsequently the sapwood volume would represent a comparatively small water reservoir. These physiological conditions would predispose the upper crown to bark beetle attack.

Furthermore, comparative olfactory studies using sections from various stem heights indicated that olfactometers baited with billets from the upper crown captured the largest portion of the responding beetles. A precedent studied (48) demonstrated that Ips confusus will accumulate at a source with the highest pheromone concentration; therefore, "pioneer" beetles successfully attacking the crown would elicit a comparatively larger attack density than a similar number of beetles attacking the stem base. The consequence of the above factors would seem to explain the phenomena of top kill in the large d.b.h. classes in ponderosa pine.

The presence of interspecific differences in phloem thickness, measured at breast height, can not be related to the dissimilitudes in response patterns. The present data indicated that an increased consumption of dead bark, i.e., when the subcortical tissue is thinner than the average dorsal-ventral dimension of a male beetle, did not alter the apparent level of pheromone synthesis. As suggested by these studies, differences in response behavior can be related to trees with a comparatively

high osmotic value of the expressed phloem sap, irrespective of thickness. An extremely high o.p., i.e., 15 atmospheres, does not appear to enhance the attractant synthesis. These data point to the difficulty in establishing definitive values which are meaningful in assessing the comparative suitability of ponderosa pine. Rudinsky (109) in drawing inferences to this difficulty stated, "...the wide variation of osmotic pressures obtainable from individual trees, from trees on different sites and throughout a season, does not permit the establishment of critical, species-specific values...."

Although proposing discrete o.p. values which reflect upon the phloem suitability is precluded through the o.p. variability, a qualified comment can be made concerning the polarity of this clinical indicator.

In reference to pheromone synthesis, subcortical tissue with osmotic values in the range of 12 to 15 atmospheres appears to be nutritionally better than tissue possessing osmotic values from seven to 11 atmospheres. Therefore, ponderosa pine, which are apparently "thrifty," i.e., with a full crown, rapid increment increase, high o.e.p. and o.p., may effectuate a significant increase in the rapidity and magnitude of the colonization by Ips confusus once the tree's resistance (o.e.p.) has been rendered ineffective (i.e., drought, fire, etc.). An extension of this conjecture

would suggest that trees showing no signs of decadency are potentially better material for colonization than suppressed decadent individuals.

Vité (128) concluded that the resistance of ponderosa pine to bark beetle attack was not solely a physical expression of oleoresin. Other workers (2, 26 and 45) demonstrated that several conifer infesting beetles are repelled by several of the volatile fractions of oleoresin. A preliminary study corroborated these findings and suggested that several of the mono-turpenes can act as repellents to Ips confusus.

Field olfactory studies indicated that Ips confusus are repelled by alpha and beta pinene, myrecene, and delta 3 carene, all common constituents of ponderosa pine turpentine (89). Response to olfactometers containing fresh billets with recently forced males can be markedly reduced if the exposed xylem is covered with exuded oleoresin. The degree of olfactory inhibition is dependent upon the concentration of the volatile turpenes. Olfactory repellency is considerably more acute in the presence of a beaker containing 100 mls. of oleoresin than in the presence of a similar amount in an Erlenmeyer flask. The larger orifice of the beaker facilitates a higher evaporation rate which apparently increases olfactory interference.

In view of these observations, it seems plausible

to suggest that Ceratocystis ips is instrumental in the total expression of the Ips confusus pheromone. The ultimate colonization of a new host is dependent upon two separate phases; the initial attack or hose selection phase followed by a mass attack or concentration phase. The success of the colonization is jeopardized by the mechanical interference of oleoresin plus the repellent properties of its volatile fractions. Therefore, a prophylaxis such as an embolism caused by hyphal permeation of resin channels would effectively eliminate the physical and volatile interference of exuding oleoresin. The elimination of oleoresin augments the success of the initial attack, thereby assuring the ensuing mass attack.

SUMMARY

1. The osmotic values of expressed phloem sap were followed from May through September in three trees with zero o.e.p. and three trees with an o.e.p. in excess of 166 p.s.i. The initial determinations indicated that high pressure trees had an o.p. in excess of 11 atm. while the low pressure trees had an o.p. less than 8.5 atm. This distinction was not clear throughout the following four months.

2. Measurements of the phloem thickness in six trees indicated an increase in dimensions from the butt to mid crown whereafter the phloem decreased in thickness. No salient correlation could be determined between phloem dimension and o.e.p.

3. Relative turgidity measurements of ponderosa pine phloem do not appear applicable as an indicator of the trees' physiological profile. A wide variation in the amount of soluble materials precludes the establishment of cogent categories.

4. Inoculation studies with the blue-staining fungus, Ceratocystis ips on standing and cut trees suggest that the microorganism is aiding colonization and brood development of Ips confusus in no other way than through reducing moisture movement and oleoresin exudation in the outer sap wood. The host resistance, as manifested by

the o.e.p., can be of sufficient magnitude to discourage the combined efforts of both organisms.

5. When offered a choice of four humidity gradients, Ips confusus preferred the higher moisture concentration, provided the relative humidity of the ambient air was low. These findings prompted the conclusion that olfactory resolution, by responding female Ips confusus, can be increased by conducting the studies in a water saturated atmosphere.

6. The apparent level of pheromone synthesis was enhanced when certain sugars were added to the diet of male Ips confusus. Of the carbohydrates tested, including maltose, sucrose, fructose, glucose and starch, only those carbohydrates, with the exception of starch, containing glucose moieties were effectual as a dietary supplement. Glucose per se was apparently prominent in educing the pheromone synthesis. Variance of these data precluded an unqualified implication of glucose as the single most important dietary element.

7. Data from studies on the effect of extracting the homogenized phloem diet with acetone and ethyl ether did not implicate a fat-soluble compound as responsible for the pheromone synthesis. These findings imply that the dietary element is water soluble and, conversely, lend substance to the implication of phloem sugar.

8. Response dissimilitudes in field olfactory studies were related to the osmotic values of the expressed phloem sap. Field olfactometers baited with billets from trees with osmotic values in excess of 11 atmospheres were consistently more efficient in capturing responding Ips confusus. Trees with phloem low in non-electrolytes appeared to represent a substrate which was less favorable as a dietary media for pheromone synthesis.

9. The preceding conclusion was demonstrated experimentally by severing the sieve elements of ponderosa pine for various periods of time. After one month and one year significant increases were detected in the o.p. of the phloem sap distal to the girdle. These differences were also manifested when field olfactometers were baited with billets distal and proximal to the wound. As evident by the differences in response patterns, male Ips confusus were capable of eliciting a greater olfactory stimuli while feeding in billets taken distal to a girdle.

10. An extensive infection by Cronartium harknessii may also result in intraspecific differences in host suitability which can be demonstrated by response disparities. Extended development of the fungus led to phloem severance which in turn was manifested by a parallel in olfactory results as obtained with girdled trees.

11. For the most part, all data obtained from the field test on host differences were corroborated by laboratory studies. This was accomplished by forcing ten male Ips confusus into three 1/2-inch discs taken from the various trees. Frass was subsequently collected and threshold studies conducted with the laboratory olfactometers.

12. Dissimilitudes in response patterns were also related to the various heights of the stem from which the billets were obtained. Consistent in two separate studies, male Ips confusus forced into billets from the upper stem portion were capable of eliciting a greater response than males in the butt portion. Phloem tissue reaches its greatest dimension at mid crown.

13. The theory was tested that beetles forced to ingest nutritionally poor material (dead bark) may in turn show a marked reduction in their ability to synthesize the attractant principle. This theory was examined by forcing males into billets with phloem thinner than their average dorsal-ventral dimension (.056 inches). Comparative studies of trees with a phloem thickness of .060, .034 and .026 inches did not indicate that trees with phloem in excess of .056 inches were superior as host material. Instead, the data demonstrated a close correlation between the response pattern and the osmotic values of the expressed phloem sap.

14. As a corollary of the preceding experiment, data were obtained which demonstrated the influence of ponderosa pine oleoresin in the presence of the Ips confusus pheromone. Volatilized oleoresin appeared to have a strong repellent action when examined in the presence of the pheromone. Olfactory resolution was reduced when the exposed surfaces of the billets were coated with exuding oleoresin. This interference was eliminated by washing the exposed xylem with ethyl alcohol and then applying a coat of molten paraffin.

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APPENDIX

Appendix I.

Analysis of variance on female Ips confusus' preference for four humidity gradients in a laboratory olfactometer with a 38 percent relative humidity air stream

Source of variation	D.F.	Sum of squares	Mean square	F
Total	23	663.959	28.8677	
Replications	5	27.709	5.5418	.66
Treatments	3	511.459	170.4863	20.94***
Error	15	124.791	8.3194	

*** Significant at the .5% level

Appendix II.

Analysis of variance on female Ips confusus' preference for four humidity gradients in a laboratory olfactometer with a 0 percent relative humidity air stream

Source of variation	D.F.	Sum of squares	Mean square	F
Total	19	124.2	6.536	
Replications	4	13.7	3.425	1.26
Treatments	3	77.8	25.933	9.53***
Error	12	32.7	2.72	

***Significant at the .5% level

Appendix III.

Factorial analysis of variance of female Ips confusus preference to various materials in laboratory olfactometers with air streams of 100 percent, 35 percent, 0 percent relative humidity

Source of variation	D.F.	Sum of squares	Mean square	F
Total	107	2.1769	.0203	
Olfactometers(1)	2	.0120	.0060	.70
Replications(2)	2	.0172	.0086	.10
Treatments(3)	3	.6477	.2159	25.40***
Sectors(4)	2	.1175	.0587	6.90
1x2	4	.0608	.0152	1.79
1x3	6	.0833	.0138	1.65
1x4	4	.1146	.0286	3.36*
2x3	6	.0299	.0049	.58
2x4	4	.0203	.0050	.59
3x4	6	.2247	.0374	4.40**
1x2x3	12	.1057	.0088	1.04
1x2x4	8	.0222	.0027	.32
1x3x4	12	.0627	.0052	.61
2x3x4	12	.4541	.0378	4.45**
Residual (Error)	24	.2042	.0085	

* Significant at 5 percent level

** Significant at 1 percent level

*** Significant at .5 percent level

Appendix IV.

Factorial analysis of variance of the comparative response by females elicited by males feeding in homogenized phloem supplemented with three different sugars. April 17, 1963 study

Source of variation	D.F.	Sum of squares	Mean square	F
Total	107	1.5146	.0412	
Replications(1)	8	.0971	.0121	1.33
Treatments(2)	3	.2223	.0741	8.14***
Sectors(3)	2	.0935	.0467	5.13**
1x2	24	.2792	.0116	1.27
1x3	16	.2659	.0166	1.82
2x3	6	.1154	.0192	2.11
Residual(Error)	48	.4412	.0091	

** Significant at 1% level

*** Significant at .5% level

Appendix V.

Factorial analysis of variance of the comparative response by females elicited by males feeding in homogenized phloem supplemented with three different sugars. August 29, 1963 study

Source of variaton	D.F.	Sum of squares	Mean square	F.
Total	107	.7916	.0074	
Replications(1)	8	.0269	.0033	.80
Treatments(2)	3	.1049	.0349	8.51***
Sectors(3)	2	.0802	.0401	9.78***
1x2	24	.1162	.0048	1.17
1x3	16	.0617	.0038	.93
2x3	6	.2026	.0337	8.22***
Residual(Error)	48	.1989	.0041	

*** Significant at .5% level

Appendix VI.

Factorial analysis of variance of the comparative response by females elicited by males feeding in homogenized phloem supplemented with three different carbohydrates, e.g., starch, glucose, and sucrose

Source of variation	D.F.	Sum of squares	Mean square	F
Total	119	20.1712	.1695	
Olfactometer(1)	1	.0367	.0367	.42
Treatments(2)	3	1.3069	.4356	4.98**
Replication(3)	4	.2425	.0606	.69
Sectors(4)	2	.9620	.4810	5.50*
1x2	3	.0663	.0221	.25
1x3	4	.1512	.0378	.43
1x4	2	.3020	.1510	1.73
2x3	12	1.8935	.1577	1.80
2x4	6	4.0233	.6705	7.67***
3x4	8	2.0080	.2510	2.87
1x2x3	12	.7941	.0661	.76
1x2x4	6	1.1220	.1870	2.14
1x3x4	8	1.1463	.1432	1.64
2x3x4	24	4.0200	.1675	1.92
Residual(Error)	24	2.0974	.0874	

* Significant at 5% level

** Significant at 1% level

*** Significant at .5% level

Appendix VII.

Factorial analysis of variance of the comparative response by females elicited by males feeding in homogenized phloem supplemented with three different sugars, e.g., raffinose, glucose, and sucrose

Source of variation	D.F.	Sum of squares	Mean square	F
Total	71	1.0209	.0143	
Replications(1)	5	.0275	.0055	1.96
Treatments(2)	3	.2401	.0800	28.75***
Sectors(3)	2	.2601	.1300	46.43***
1x2	15	.0452	.0030	.10
1x3	10	.1325	.0132	4.72***
2x3	6	.2305	.0384	13.71***
Residual(Error)	30	.0850	.0028	

*** Significant at the .5% level

Appendix VIII.

Factorial analysis of variance of the comparative response by female Ips confusus elicited by males feeding in homogenized phloem extracted with acetone and water and supplemented with three different solutions, e.g., one molar glucose, distilled water, and water used in the homogenizing treatment

Source of variation	D.F.	Sum of squares	Mean square	F
Total	95	1.5229	.0610	
Replications(1)	7	.0554	.0079	1.32
Treatments(2)	3	.1063	.0354	5.90***
Sectors(3)	2	.0704	.0352	5.88**
1x2	21	.1218	.0058	.97
1x3	14	.2169	.0154	2.57**
2x3	6	.6966	.1161	19.35***
Residual(Error)	42	.2555	.0060	

** Significant at the 1% level

*** Significant at the .5% level

Appendix IX.

Factorial analysis of variance of the comparative response to field olfactometers baited with billets of four physiological types, e.g., from trees with high o.p. and low o.p. and the portions above and below the site of infection caused by Cronartium harknessii

Source of variation	D.F.	Sum of squares	Mean square	F
Total	791	1112299.328	1406.194	
Treatments[1] (1)	3	48829.873	16276.624	13.65***
Days[2] (2)	10	20297.783	2029.778	3.01***
Time[2] (3)	5	22747.305	4549.461	6.75***
Sectors[2] (4)	2	7329.935	3664.968	5.44**
1x2	30	114635.245	3821.175	N.I.[3]
1x3	15	20987.526	1399.168	N.I.
1x4	6	10525.274	1754.212	N.I.
2x3	50	175894.652	3517.893	5.22***
2x4	20	81523.763	4076.1881	6.05***
3x4	10	5785.438	578.544	.86
1x2x3	150	108254.559	721.697	N.I.
1x2x4	60	39455.426	657.590	N.I.
1x3x4	30	30879.633	1029.321	N.I.
2x3x4	100	67363.430	673.634	N.D.[4]
Residual(Error)	300	357789.500	1192.631	

[1] Fixed effect

[2] Random effect

[3] Interaction component assumed to equal zero

[4] A denominator for computing the F ratio is not available in 1 fixed-3 random effect model

** Significant at the 1% level

*** Significant at the .5% level

Appendix X.

Factorial analysis of variance of the comparative response to field olfactometers baited with billets removed from the distal and proximal perimeters of a canker caused by Cronartium harknessii

Source of variation	D.F.	Sum of squares	Mean square	F
Total	95	245.635	2.586	
Treatments[1] (1)	1	1.365	1.365	2.19
Days[2] (2)	1	1.162	1.162	.91
Time[2] (3)	5	72.517	14.503	11.40***
Sector[2] (4)	3	58.089	19.363	15.22***
1x2	1	.7150	.715	N.I.[3]
1x3	5	.8747	.175	N.I.
1x4	3	24.486	8.162	N.I.
2x3	5	2.728	.546	.43
2x4	3	4.970	1.657	1.30
3x4	15	36.940	2.463	1.94
1x2x3	5	.370	.074	N.I.
1x2x4	3	9.076	3.025	N.I.
1x3x4	15	3.899	.260	N.I.
2x3x4	15	19.080	1.272	N.D.[4]
Residual(Error)	15	9.3641	.624	

[1] Fixed effect

[2] Random effect

[3] Interaction component assumed to equal zero

[4] A denominator for computing the F ratio is not available in 1 fixed-3 random effect model

*** Significant at the .5% level

Appendix XI.

Factorial analysis of variance of the comparative response to field olfactometers baited with billets taken distal and proximal to a one-month-old girdle (tree 1a and 1b)

Source of variation	D.F.	Sum of squares	Mean square	F
Total	79	282.881	1.049	
Treatment[1] (1)	1	7.782	7.782	7.71*
Days[2] (2)	1	16.907	16.907	7.58*
Time[2] (3)	4	38.955	9.739	4.37*
Sector[2] (4)	3	52.236	17.412	7.81***
1x2	1	1.288	1.288	N.I.[3]
1x3	4	3.743	.936	N.I.
1x4	3	15.128	5.043	N.I.
2x3	4	33.942	8.486	3.81
2x4	3	14.820	4.940	2.22
1x2x3	4	2.060	.515	N.I.
1x2x4	3	2.825	.942	N.I.
1x3x4	12	12.031	1.002	N.I.
2x3x4	12	26.742	2.2284	N.D.[4]
Residual (Error)	12	12.114	1.0095	

[1] Fixed effect

[2] Random effect

[3] Interaction component assumed to equal zero

[4] A denominator for computing the F ratio is not available in 1 fixed-3 random effect model

* Significant at the 5% level

*** Significant at the .5% level

Appendix XII.

Factorial analysis of variance of the comparative response to field olfactometers baited with billets taken distal and proximal to a one-month-old girdle (tree 2a and 2b)

Source of variation	D.F.	Sum of squares	Mean square	F
Total	71	15.380	.217	
Treatment[1] (1)	1	1.326	1.326	91.45***
Days[2] (2)	1	.642	.642	1.96
Time[2] (3)	5	7.275	1.455	44.49***
Sector[2] (4)	2	2.628	1.314	40.18***
1x2	1	.005	.005	N.I.[3]
1x3	5	.839	.168	N.I.
1x4	2	.173	.086	N.I.
2x3	5	.674	.135	4.13*
2x4	2	.007	.003	.10
3x4	10	.970	.097	2.96*
1x2x3	5	.060	.012	N.I.
1x2x4	2	.078	.039	N.I.
1x3x4	10	.232	.023	N.I.
2x3x4	10	.327	.0327	N.D.[4]
Residual(Error)	10	.1449	.0145	

[1] Fixed effect

[2] Random effect

[3] Interaction component assumed to equal zero

[4] A denominator for computing the F ratio is not available in 1 fixed-3 random effect model

* Significant at the 5% level

*** Significant at the .5% level

Appendix XIII.

Factorial analysis of variance of the comparative response to field olfactometers baited with billets taken distal and proximal to a one-year-old girdle (tree 3a and 3b)

Source of variation	D.F.	Sum of squares	Mean square	F
Total	95	258.273	2.719	
Treatment[1] (1)	1	28.017	28.017	66.30***
Days[2] (2)	1	.051	.051	.04
Time[2] (3)	5	87.725	17.545	13.88***
Sector[2] (4)	3	30.727	10.242	8.10***
1x2	1	.345	.345	N.I.[3]
1x3	5	14.580	2.916	N.I.
1x4	3	10.306	3.435	N.I.
2x3	5	10.138	2.028	1.60
2x4	3	12.207	4.069	3.22
3x4	15	16.301	1.087	.86
1x2x3	5	2.820	.564	N.I.
1x2x4	3	6.302	2.101	N.I.
1x3x4	15	13.453	.897	N.I.
2x3x4	15	18.962	1.2641	N.D.[4]
Residual (Error)	15	6.3394	.4226	

[1] Fixed effect

[2] Random effect

[3] Interaction component assumed to equal zero

[4] A denominator for computing the F ratio is not available in 1 fixed-3 random effect model

*** Significant at the .5% level

Appendix XIV.

Factorial analysis of variance of the comparative response to field olfactometers baited with billets taken distal and proximal to a one-year-old girdle (tree 4a and 4b)

Source of variation	D.F.	Sum of squares	Mean square	F
Total	95	57.864	.609	
Treatment[1] (1)	1	18.288	18.288	142.99***
Days[2] (2)	1	.788	.788	6.45*
Time[2] (3)	5	10.801	2.160	17.68***
Sector[2] (4)	3	7.506	2.502	20.47***
1x2	1	.364	.364	N.I.[3]
1x3	5	3.969	.794	N.I.
1x4	3	2.190	.730	N.I.
2x3	5	1.235	.247	2.02
2x4	3	.646	.215	1.76
3x4	15	4.209	.281	2.29
1x2x3	5	.388	.078	N.I.
1x2x4	3	.608	.203	N.I.
1x3x4	15	3.121	.208	N.I.
2x3x4	15	1.833	.1222	N.D.[4]
Residual(Error)	15	1.919	.1279	

[1] Fixed effect

[2] Random effect

[3] Interaction component assumed to equal zero

[4] A denominator for computing the F ratio is not available in 1 fixed-3 random effect model

* Significant at the 5% level

*** Significant at the .5% level

Appendix XV.

Factorial analysis of variance of the comparative response to field olfactometers baited with billets taken distal and proximal to a six-year-old girdle (tree 5a and 5b)

Source of variation	D.F.	Sum of squares	Mean square	F
Total	95	737.928	7.768	
Treatment[1] (1)	1	3.651	3.651	6.40*
Days[2] (2)	1	66.465	66.465	22.02***
Time[2] (3)	5	166.557	33.311	11.03***
Sector[2] (4)	3	152.939	50.980	16.89***
1x2	1	.140	.140	N.I.[3]
1x3	5	3.180	.636	N.I.
1x4	3	23.920	7.973	N.I.
2x3	5	38.533	7.707	2.55
2x4	3	45.116	15.038	4.98*
3x4	15	147.896	9.860	3.27
1x2x3	5	1.710	.342	N.I.
1x2x4	3	6.446	2.148	N.I.
1x3x4	15	20.545	1.836	N.I.
2x3x4	15	45.272	3.0181	N.D.[4]
Residual (Error)	15	8.558	.5705	

[1] Fixed effect

[2] Random effect

[3] Interaction component assumed to equal zero

[4] A denominator for computing the F ratio is not available in 1 fixed-3 random effect model

* Significant at the 5% level

*** Significant at the .5% level

Appendix XVI.

Factorial analysis of variance of the comparative response by female *Ips confusus* to frass produced by males feeding in 3-1/2 inch discs of four physiological types, e.g., trees with high o.p., low o.p., and above and below a stem canker caused by *Cronartium harknessii*

Source of variation	D.F.	Sum of squares	Mean square	F
Total	143	2.964	.0207	12.00***
Days(1)	1	.0780	.0780	12.00***
Replications(2)	5	.0029	.0005	.08
Treatment(3)	3	.5401	.1800	27.69***
Sector(4)	2	.4434	.2217	34.11***
1x2	5	.0274	.0054	.83
1x3	3	.2368	.0789	12.14***
1x4	2	.0107	.0053	.82
2x3	15	.0595	.0039	.60
2x4	10	.2048	.0204	3.14**
3x4	6	.0899	.0149	2.29*
1x2x3	15	.1023	.0068	1.05
1x2x4	10	.1849	.0184	2.83*
1x3x4	6	.6159	.1026	15.78***
2x3x4	30	.1701	.0056	.86
Residual(Error)	30	.1972	.0065	

* Significant at the 5% level

** Significant at the 1% level

*** Significant at the .5% level

Appendix XVII.

Factorial analysis of variance of the comparative response to field olfactometers baited with billets taken at four different stem heights (tree 1)

Source of variation	D.F.	Sum of squares	Mean square	F
Total	215	1332.450	6.197	
Height[1/] (1)	3	208.083	69.361	20.16***
Days[2/] (2)	2	3.883	1.942	.45
Time[2/] (3)	5	54.171	10.834	2.54
Sector[2/] (4)	2	336.462	168.231	39.40***
1x2	6	1.077	.178	N.I.[3/]
1x3	15	115.061	2.671	N.I.
1x4	6	135.204	22.534	N.I.
2x3	10	34.642	3.464	.81
2x4	4	6.585	1.646	.38
3x4	10	30.264	3.026	.71
1x2x3	30	38.384	1.279	N.I.
1x2x4	12	2.615	.218	N.I.
1x3x4	30	74.168	2.472	N.I.
2x3x4	20	85.391	4.270	N.D.[4/]
Residual(Error)	60	206.460	3.441	

[1/] Fixed effect

[2/] Random effect

[3/] Interaction component assumed to equal zero

[4/] A denominator for computing the F ratio is not available in 1 fixed-3 random effect model

*** Significant at the .5% level

Appendix XVIII.

Factorial analysis of variance of the comparative response to field olfactometers baited with billets taken at four different stem heights (tree 2)

Source of variation	D.F.	Sum of squares	Mean square	F
Total	215	575.009	2.674	
Height[1] (1)	3	29.828	9.942	12.52***
Days[2] (2)	2	83.232	41.616	14.27***
Time[2] (3)	5	73.184	14.637	5.02***
Sector[2] (4)	2	27.977	13.989	4.79*
1x2	6	10.326	1.721	N.I.[3]
1x3	15	14.152	.943	N.I.
1x4	6	13.489	2.248	N.I.
2x3	10	79.064	7.906	2.71*
2x4	4	19.500	4.875	1.67
3x4	10	31.054	3.105	1.06
1x2x3	30	37.816	1.260	N.I.
1x2x4	12	14.637	1.220	N.I.
1x3x4	30	34.767	1.159	N.I.
2x3x4	20	58.338	2.9168	N.D.[4]
Residual(Error)	60	47.646	.7940	

[1] Fixed effect

[2] Random effect

[3] Interaction component assumed to equal zero

[4] A denominator for computing the F ratio is not available in 1 fixed-3 random effect model

* Significant at the 5% level

*** Significant at the .5% level

Appendix XIX.

Factorial analysis of variance of the comparative response to field olfactometers baited with billets and slabs taken from trees with four different phloem dimensions, e.g., .146, .060, .034, and .026 inches

Source of variation	D.F.	Sum of squares	Mean square	F
Total	287	438214.29	1526.88	
Treatments[1] (1)	1	5416.67	5416.67	10.34***
Phloem[1] (2)	3	26863.25	8954.42	17.10***
Days[2] (3)	1	6450.58	6450.58	5.50**
Time[2] (4)	5	62421.88	12484.38	10.65***
Sector[2] (5)	2	24654.58	12327.29	10.51**
1x2	1	11259.99	3753.33	7.17***
1x3	1	4117.79	4117.79	N.I.[3]
1x4	5	13250.65	2650.13	N.I.
1x5	2	26638.80	13319.40	N.I.
2x3	3	4071.52	13517.17	N.I.
2x4	15	14456.94	963.80	N.I.
2x5	6	11353.20	1892.20	N.I.
3x4	5	14622.40	2924.48	2.49
3x5	2	4900.64	2450.32	2.09
4x5	10	17021.76	1702.18	1.45
3x4x5	10	11724.18	1172.41846	N.D.[4]
Residual(Error)	30	15705.34	523.51119	
Second order interactions[5]				
Third order interactions[5]				

[1] Fixed effect

[2] Random effect

[3] Interaction component assumed to equal zero

[4] A denominator for computing the F ratio is not available in 2 fixed-3 random effect model

[5] Interactions omitted

** Significant at 1% level; ***significant at .5% level.