RELATIONS OF THE BLUE STAIN FUNGUS,
CERATOCYSTIS IPS (RUMBOLD)
C. MOREAU, TO *IPS* BARK BEETLES
(COLEOPTERA: SCOLYTIDAE)
OCCURRING IN FLORIDA

By
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INTRODUCTION

The association between blue stain fungi, particularly of the genus Ceratocystis Ellis and Halstead, and scolytid bark beetles in the genera Dendroctonus Erichson and Ips DeGeer has long been known. Because of the constant presence of blue stain fungi in the phloem and sapwood of trees attacked by bark beetles, early workers generally assumed the fungi were commonly introduced under the bark by insects. Leach, Orr, and Christensen (1934) used a series of caged and uncaged logs of red pine, Pinus resinosa Ait., to prove that Ips grandicollis (Elchh.) and Ips pini (Say) transmitted the blue stain fungus, Ceratocystis ips (Rumbold) C. Moreau. They concluded that certain blue stain fungi, such as C. ips, were rarely, if ever, transmitted in any other way.

Most previous research on blue stain fungi and bark beetles was directed at determining the species of fungi associated with various bark beetles and the effect of the fungi on the host trees and their wood products. No comprehensive study was made of the relationship between the organisms involved, and the nature of the association was open to considerable question. Craighead (1928), Leach (1940), Leach et al. (1934) and Nelson (1934) considered the association to be one of mutualism, with the fungi benefiting from transmission by the beetles to a suitable host and the insects benefiting from creation of a more favorable environment for brood development by

action of the fungi on the wood and inner bark. Holst (1937) in the United States and Grosmann (1930) in Germany reared certain bark beetles from egg to adult free of their blue stain associates and concluded the fungi were not essential for development within a single generation of the insects. Hetrick (1949) observed an infestation of the southern pine beetle, Dendroctonus frontalis Zimm., in which the trees showed no signs of blue staining. Since beetle brood development appeared to be normal, he concluded that blue stain organisms were not essential for successful attack by the beetles.

In light of the uncertainty of the exact nature of the blue stain fungus-bark beetle association, a project was initiated to study the Ips bark beetles occurring in Florida; Ips avulsus (Elchh.), Ips calligraphus (Germ.), and Ips grandicollis (Elchh.), and their blue stain fungus associate, Ceratocystis ips (Rumbold) C. Moreau. The principal objective of the study was to compare development of all stages of the beetles in one or more generations in the presence or absence of the fungus. The results of this investigation together with observations on the biology and behavior of the beetles are reported herein.

LITERATURE REVIEW

Thatcher (1960) reviewed current knowledge of the bark beetles attacking southern pines which included the lps species occurring in Florida. These beetles have not been studied intensively and no detailed data were available on their biology and habits. A discussion of blue stain in timber was recently presented by Findlay (1959a, b) in which he covered the fungi involved and their effect on strength and quality of wood, dissemination, prevention, and control.

Bark beetle-associated blue stain fungi have been described in several genera, but most have belonged to the genus Ceratocystis. Species of Graphium and Leptographium, many of which were shown to be imperfect forms of Ceratocystis, and Tuberculariella lps Leach, Orr, and Christensen also have been found to cause blue stain (Mathre, 1964a). The present study was restricted to the Ceratocystis associates of lps species bark beetles in Florida. Hunt (1956) revised the genus Ceratocystis, and all previous citations from the literature have been given in terms of his monograph.

Transmission of Ceratocystis Species

The bluing of timber was first described by Hartig in 1878 (Hartig, 1894). He recognized the fungal nature of the stain, Ceratocystis piliifera (Fries) C. Moreau, and noted that the fungus

commonly occurred in trees attacked by insects. Von Schrenk (1903) studied blue stain fungi in ponderosa pine, Pinus ponderosa Laws, attacked by Dendroctonus ponderosae Hopk. He isolated Ceratocystis piliifera from the galleries of the beetle which suggested the beetle transmitted the fungus. Von Schrenk was unable to isolate the fungus from the intestinal tract and feces of the beetle, however, and discounted the probability of insect transmission. He concluded that the spores of the fungus were distributed by the wind and entered the region between the bark and wood through holes made by the beetles.

Little attention was given the blue stain fungus-bark beetle association until Craighead (1928) suggested that the fungi were transmitted by bark beetles. Supported by isolations of Ceratocystis species from wood adjacent to beetle galleries and directly from the beetles, subsequently workers (Bakshi, 1950; Bramble and Holst, 1935, 1940; Davidson, 1935, 1955, 1958; Ellis, 1939; Grosmann, 1930; Leach et al., 1934; Nelson, 1934; Nelson and Beal, 1929; Robinson, 1962; Robinson-Jeffery and Grinchenko, 1964; Rumbold, 1929, 1931, 1936, 1941; Verrall, 1941; and others) generally assumed that bark beetles played an important role in dissemination of blue stain fungi. Rumbold (1931) showed that C. piliifera was a secondary invader. She was of the opinion that wood used by Von Schrenk (1903) to start his cultures was too old, and the primary fungus, probably Ceratocystis minor (Hedgc.) Hunt, had lost its vitality by the time the wood reached the laboratory. Grosmann (1930) stated that Von Schrenk's failure to isolate the fungus from the beetle could

be explained in that he used liquid agar which, owing to its high temperature of solidification, was too hot at the time of inoculation. She also added that Von Schrenk had obviously neglected to prevent bacterial growth by the addition of acid to the culture medium and to punctually isolate the germinating fungus spores. Grosmann noted that even though no exact proof had been presented, repeated observations that scolytids appeared mostly in association with blue stain fungi led to the recognition that bark beetles played a significant role as transmitters of blue stain fungi.

Leach et al. (1934) studied the interrelationships of bark beetles and blue stain fungi in felled red pine, Pinus resinosa Alt., and demonstrated transmission of Ceratocystis ips (Rumbold) C. Moreau by ips grandicollis (Elchh.) and ips pinl (Say). In a series of caged and uncaged logs with sealed and unsealed ends, C. ips occurred only in logs attacked by the bark beetles. The fungus was abundant in all uncaged logs and absent from the caged logs except when a few beetles accidentally gained entrance. In logs lightly attacked by the beetles, the fungus was limited to the immediate vicinity of the beetle tunnels. Histological preparations of adult beetles revealed ascospores of the fungus adhering in clumps to various parts of the body wall and distributed throughout the intestinal tract. Fragments of perithecia were also found in the gut, and examination of fecal pellets showed viable ascospores of the fungus to be present. In view of their findings, Leach et al. (1934) concluded that these bark beetles universally introduced blue stain fungi into logs and trees.

Not all Ceratocystis species have been found to be disseminated by bark beetles. Verrall (1941) studied dissemination of various blue stain fungi in lumber yards in Louisiana and Mississippi and found air currents, insects, milling machinery, and rainwater to be disseminating agents. C. Ips was occasionally isolated from the air, but Verrall stated Ips species bark beetles were the most important transporters of the fungus since he isolated C. Ips from 62 per cent of the bark beetles he captured in the yards. Leach et al. (1934) also presented evidence that mites associated with bark beetles often carried C. Ips spores on their bodies and distributed the spores through the beetle gallery system.

Specificity of Ceratocystis spp. - Bark Beetle Association

Depending upon their association with bark beetles, Mathiesen (1950) divided the Swedish Ceratocystis species into the following types: (1) those species spread primarily by wind and only rarely by bark beetles: e.g., C. coerulescens (Münch) Bakshi, C. floccosa (Mathiesen) Hunt, C. pilifera, and C. pluriannulata (Hedgc.) C. Moreau; (2) those species associated with several bark beetles usually in older material, but also spread by wind: e.g., C. minor, C. minuta (Slem.) Hunt, C. piceae (Münch) Bakshi, and C. polonicum (Slem.) C. Moreau; and (3) those always associated with certain species of bark beetles: e.g., C. brunneo-ciliata (Mathiesen-K.) Hunt with Ips sexdentatus Boern., C. cana (Münch) C. Moreau with Blastophagus minor Hart, and C. clavata (Mathiesen) Hunt with Ips acuminatus Gyll. In North America, Dendroctonus and Ips species

have been considered the most important bark beetles of coniferous trees. Several Ceratocystis species have been found associated with Dendroctonus bark beetles (Bramble and Holst, 1940; Davidson, 1955, 1958; Mathre, 1964a; Robinson, 1962; Robinson-Jeffrey and Grinchenko, 1964; and Rumbold, 1931, 1936, 1941), but C. minor was the most common found species. Ceratocystis ips has been the only species of the genus consistently associated with ips bark beetles.

Ceratocystis ips was described by Rumbold (1931) as an associate of ips calligraphus and ips grandicollis. The fungus was also found in association with ips avulsus (Rumbold, 1931), ips confusus (Lec.) (Mathre, 1964a), ips emarginatus (Lec.) (Mathre, 1964a; Rumbold, 1936), ips integer (Elchh.) (Rumbold, 1936), ips lecontei (Lec.) (Ellis, 1939), ips oregoni (Elchh.) (Mathre, 1964a; Rumbold, 1936), ips pini (Leach et al., 1934), ips plagiographus (Lec.) (Rumbold, 1936) and ips ponderosa Sw. (Mathre, 1964a). C. ips was also found associated with Orthotomicus caelatus (Elchh.) (Verrall, 1941), Dendroctonus valens Lec. and Dendroctonus ponderosa (Mathre, 1964a) in this country. In addition to North America, C. ips was reported in association with ips sexdentatus in Germany (Francke-Grosman, 1963) and Poland (Siemaszko, 1939), ips species in Japan (Nisikado and Yamauti, 1933), and Orthotomicus proximus (Elchh.) in Sweden (Mathiesen-Käärrik, 1960). Certain ips species in Europe were found to be associated with several Ceratocystis species other than C. ips, however.

Characteristics of Bark Beetle - Associated Ceratocystis Species

The blue stain fungi associated with bark beetles characteristically have conidia and ascospores covered with a sticky mucilage: a characteristic associated with adherence of these reproductive bodies to the body wall of the insects. This trait has not been observed in wind-borne Ceratocystis species. Leach et al. (1934) showed the sticky covering also protected the spores as they passed through the beetles' digestive tract. Mathieson-Käärlik (1960) demonstrated that the sticky layer rendered the spores resistant to desiccation. She found the spores remained viable for several months on beetles in hibernation.

The bark beetle associated Ceratocystis have been shown to be ecologically adapted to their vectors. Mathieson-Käärlik (1960) and Grosmann (1930) studied the development of certain European bark beetles and found developmental time for the fungi corresponded very closely with that of the beetles. Leach et al. (1934) made similar observations in the United States on C. Ips in association with Ips grandicollis and Ips pinl. The fungus began to grow immediately after introduction by the beetles and was isolated from wood near the galleries within a week. Isolation was possible before visible signs of staining were present. The fungus rapidly spread radially and longitudinally from the egg gallery. Tangential growth was slow and took place primarily through the larval galleries. Isolations showed that the larvae moved ahead of C. Ips and very little, if any, of the fungus was consumed. Shortly after the larvae pupated the fungus began to sporulate. Perithecia and

conidia were abundant in the old egg galleries, larval tunnels, and pupal chambers. Perithecia also formed in large numbers in the inner bark not consumed by the larvae. Francke-Grosmann (1963) noted that C. ips was especially adapted to endozoic dissemination since the spores of embedded perithecia could be spread only when young beetles ate the inner bark and carried the spores in their gut to new breeding places. Newly formed ips grandicollis and ips pini adults began feeding before leaving the logs in which they had developed, and according to Leach et al. (1934) the young beetles wandered through old egg galleries and tunneled through inner bark containing embedded perithecia. Many spores were ingested or adhered to the outside of the body wall. They stated that the beetles emerged from the logs thoroughly infested with spores of the fungus.

Through studies on the physiology of certain Ceratocystis species, Mathiesen-Kaarik (1960) attempted to differentiate between the insect and non-insect associated species. She found insect associated forms to be deficient of certain vitamins while wind-blown forms were vitamin-autotrophic. The insect associated species were able to utilize a greater number of carbohydrates and showed a wider enzymatic capacity than the wind-borne species. She was not able to demonstrate consistent physiological differences between the two groups of fungi, but she stated each fungus had its own special moisture and nutritional requirements and developmental time. Mathiesen-Kaarik (1960) concluded that all these factors together might produce optimal conditions for some particular fungus

or fungi in the galleries of a specific bark beetle; other fungi might occasionally be introduced into the galleries of this insect, but were not compatible for long periods of time and would disappear in succeeding insect generations. She noted that this would fully explain the existence of non-specialized associations between certain widespread bluing fungi and a number of different but ecologically similar bark beetles.

Nature of the Ceratocystis spp. - Bark Beetle Association

The blue stain fungus-bark beetle association has been referred to as mutualism by some workers and commensalism by others. It has been clearly demonstrated in some instances that the fungi benefit from the association. Leach et al. (1934) and Mathiesen (1950) proved that certain Ceratocystis species were almost exclusively disseminated by bark beetles. Leach et al. stated that the fungi obviously benefited from dissemination by the beetles and from introduction into the inner bark of logs and susceptible trees. St. George and Beal* indicated that the action of Dendroctonus frontalis on the inner bark created conditions favorable for C. minor growth since the fungus failed to become established when insect broods failed to develop. Pathogenicity trials (Mathre, 1964b, and Nelson, 1934) with C. ips and C. minor added further

*St. George, R. A. and J. A. Beal. 1927. Progress report on the southern pine beetle (Dendroctonus frontalis Zimm.), U. S. Bur. Entomol. Plant Quar. Div. Forest Insect Invest. Ashville, N. C., (typewritten 40 pp.) (Cited from Dixon and Osgood, 1961, p. 18).

support to this assumption. The fungi were unable to infect trees when the inoculum was introduced through needle holes intended to simulate initial bark beetle attack. Grossmann (1930) noted that blue stain fungi rapidly spread longitudinally and radially in the wood and phloem from the egg galleries of the beetles, but tangential spread was slow and took place primarily through tangentially extended larval galleries in the phloem.

Benefits derived by the beetles from the association have not been clearly defined. Craighead (1928) suggested that blue stain fungi created conditions favorable for brood development and that the fungi possibly furnished essential nourishment for the beetles. Nelson (1934) found C. minor infection resulted in a reduction of the water content of the wood, and concluded the fungus was probably necessary for optimum brood development of Dendroctonus frontalis. Leach et al. (1934) stated:

... The blue-staining fungi, by inhibiting the flow of sap, in all probability, make living trees more favorable for beetle development and by aiding in decomposition of the inner bark cause it to separate from the wood, creating a more favorable environment for development of insect broods.

They pointed out that until a brood of beetles was reared in a fungus-free log, it could not be safely concluded that blue stain fungi were not necessary for normal development of the beetles.

Other workers have expressed the opinion that both blue stain fungi and bark beetles can exist and develop fully without their associates. Grossmann (1930) found both Ips typographus L. and Blastophagus piniiperda L. to be independent of their blue stain associates. She noted that larvae of the beetles moved ahead of

the spread of the fungi through the inner bark and very little, if any, fungal material was consumed by the larvae. She observed young adults of both beetles feeding on blue stain fungi, but she was unable to detect any difference in the maturation period or vigor of beetles that had or had not fed on the fungi. Holst (1937) aseptically reared Dendroctonus frontalis, Ips calligraphus and Ips grandicollis in small numbers on strips of phloem sealed between two glass plates. Since the reared insects were within size limits given by Blackman (1922), Holst concluded that blue stain fungi were not essential for development from egg to adult within a single generation. Hetrick (1949) observed a Dendroctonus frontalis infestation in Florida in which the trees showed no sign of blue staining. He stated that the trees died rapidly and normal broods developed. Based upon this and other field observations, Hetrick concluded that blue stain fungi were not essential for successful attack by the bark beetle.

Pathogenicity of Ceratocystis Associates of Bark Beetles

Craighead (1928) pointed out that the girdling action of bark beetles alone did not fully explain the rapid death of attacked trees since mechanically girdled trees usually remained green for a year or more. The constant presence of blue stain fungi in beetle-attacked trees prompted Craighead to suggest that the fungi played a role in causing death of the tree. Subsequent pathogenicity trials with certain Ceratocystis species proved Craighead's suggestion to be correct. Nelson (1934) and Nelson and Beal (1929) were able to

kill small loblolly (Pinus taeda L.), pitch (P. rigida Mill.), shortleaf (P. echinata Mill.), and Virginia (P. virginiana, Mill.) pines with C. minor. Bramble and Holst (1940) and Caird (1935) were also able to kill shortleaf pines with the fungus. Nelson (1934) inoculated trees with C. ips, but the results were inconclusive since the trees were attacked by bark beetles. Mathre (1964b) recently demonstrated that C. ips, as well as C. minor, C. monita (Rumbold) Hunt, and C. schrenkiana (Hedgc.) C. Moreau killed small ponderosa pines, P. ponderosa. In order to kill the trees, Mathre (1964b) and Nelson (1934) found it necessary to apply the inoculum over a large area which completely encircled the tree. Trees inoculated in small patches or needle holes, so as to simulate initial bark beetle attacks, were resistant to infection. Additional tests with secondary fungi associated with bark beetles; C. pilifera (Mathre, 1964a), Trichoderma spp. (Mathre, 1964a and Bramble and Holst, 1940), and Dacromyces spp. (Bramble and Holst, 1940) showed them to be non-pathogenic.

Mathre (1964b) found oleoresin exudation pressure (o.e.p.) to be a good indicator of the susceptibility of large ponderosa pines to infection by C. ips and C. minor. Trees with an o.e.p. greater than 35 p.s.i. were resistant to infection. Small trees were susceptible to infection regardless of the o.e.p., probably because of the small resin volume to the exposed surface area ratio in such trees.

Mathre (1964b) indicated the role played by C. minor in killing trees attacked by the western pine beetle, Dendroctonus brevicornis Lec., was not clear. It was shown that the beetle preferred to

attack large trees with an o.e.p. below 60 p.s.l., but was able to mass attack and kill large, high o.e.p. trees (Miller and Keen, 1960 and Vite' and Wood, 1961). Mathre speculated that in such cases, the reduction of o.e.p. was not due to C. minor since the fungus probably would not infect the trees until the o.e.p. was reduced to near zero. He added further that the trees were usually stained in scattered patches and wedges only and that water conduction was probably not stopped until the bole was completely invaded by the fungus. Ips bark beetles, on the other hand, seldom attacked trees unless the o.e.p. was near zero; a condition suitable for rapid infection by C. ips. Trees attacked by Ips species were completely stained by the fungus and water conduction was stopped. Mathre (1964b) stated "these observations suggest C. minor may not be necessary to kill trees attacked by the western pine beetle, whereas C. ips may play a role in killing trees attacked by Ips beetles."

MATERIALS AND METHODS

Fungus Isolations

Blue stain fungus isolations were made from the wood and inner bark of loblolly, Pinus taeda L.; longleaf, P. palustris Mill.; pond, P. serotina Michx.; sand, P. clausa (Chapm.) Vasey; spruce, P. glabra Walt.; and typical slash, P. elliotii var. elliotii Engelm. pines infested with one or a combination of the three species of ips bark beetles occurring in Florida. Additional isolations were made from surface sterilized eggs, larvae, pupae, and adults; non-sterilized adults; and galleries, including frass, of the three beetle species.

All isolations were made on a malt extract, yeast extract agar medium consisting of 10 grams malt extract, 2 grams yeast extract, and 20 grams agar per liter of water. Isolations from wood were made by cutting small blocks of stained wood from beetle infested host material, dipping them in 70 per cent ethyl alcohol, flaming to reduce surface contamination, cutting small chips from the blocks, and placing the chips in 2 per cent water agar. Sufficient nutrients were present in the wood chips to support fungus growth, yet yeast and bacterial contaminants were confined to the chips and immediate vicinity. After a 10-day incubation period at 24° C., a small piece of water agar bearing fungus mycelia was removed and transferred to the culture medium. Inner bark isolations were carried out as above, with the exception that surfaces were not decontaminated by dipping

in alcohol and flaming. Cultures from beetle galleries were obtained by touching a small piece of sterile agar to the spore bearing tips of perithecia extending into the galleries and placing the seeded agar on the medium. Frass plugs were teased from the egg niches and placed on the culture medium. Isolations from surface sterilized eggs, larvae, pupae, and adults and nonsterilized adults were made by crushing the specimens with sterile forceps and streaking the fragments on the surface of the medium. Surface sterilization was accomplished by washing the specimens for 5 minutes in the following modification of the sterilizing solution given by Vanderzant and Davich (1958); 0.25 grams mercuric chloride, 6.5 grams sodium chloride, 1.25 milliliters hydrochloric acid, 250 milliliters 95 per cent ethyl alcohol and 750 milliliters distilled water. The wash was followed with a 3-minute rinse in sterile water.

Frequency of *C. Ips* Transmission

Beetles used in studies on the frequency of *Ceratocystis ips* transmission were collected by two methods. *Ips calligraphus* and *Ips grandicollis* adults were collected utilizing the male attraction phenomenon (Anderson, 1948, and Wilkinson, 1964). Longleaf or loblolly pine bolts, 5 feet long and 4 to 8 inches inside bark diameter, were cut from felled trees. The bolts were transferred to an insect-tight room held at 40 to 50 per cent relative humidity and cured (dehydrated) until the phloem moisture content was approximately 100 per cent (o.d.w.). After curing, which usually required 7 days, 5 bolts were removed and placed in a 6' X 6' X 6' screen (16 mesh)

cage (Figure 1) located in a pine woods on the University of Florida Agronomy Farm. A minimum of 100 wild Ips calligraphus or Ips grandicollis males were released in the cage and allowed to infest the bolts. After a 48-hour period, the cage was visited hourly during the light period of the day, and the attracted beetles were individually collected from the outer surface of the cage and placed in 000 gelatin capsules. Ips avulsus adults were collected in 00 gelatin capsules as they emerged from infested loblolly pine slash collected in Marion County, Florida.

Slash pine bolts, 6 inches long and 3 to 5 inches in diameter, were cut from the clear portion of the bole of felled trees. Loose bark scales were removed from the bolts with a pruning knife, and the ends of the bolts were surface-decontaminated by dipping them for 30 seconds in 70 per cent alcohol containing the fungistatic agents, methyl parahydroxybenzoate and sorbic acid at rates of 1.0 per cent and 1.5 per cent, respectively. The bolts were placed in an open rack and cured for 3 days at 30° C. and 40 to 50 per cent relative humidity. Following curing, the ends of the bolts were again dipped for 30 seconds. After evaporation of the alcohol, ends of the bolts were painted with a commercially available, fungicidal and bactericidal, asphalt based pruning paint* containing 0.84 per cent metallic-copper derived from brown copper oxide.

To determine the frequency of C. ips transmission, the bolts were artificially infested with the collected beetles. "Starter

*"Seelskin Pruning Coat." Florida Agricultural Supply Company, Jacksonville, Florida.

Figure 1. Screen cage, 6' X 6' X 6', containing male infested loblolly pine bolts used to attract wild beetles.

holes" large enough to admit a given ips species, 1/16, 5/64, and 7/64 inches for ips avulsus, ips grandicollis, and ips calligraphus, respectively, were drilled obliquely into the outer bark only and care was taken not to score the phloem. Two holes were drilled per bolt, on opposing sides, and midway the length of the bolt. The drill bit was dipped in 70 per cent ethyl alcohol before boring each hole. The beetles were sexed according to the presence (female) or absence (male) of a stridulatory organ on the top of the head (Wilkinson, 1962). A single adult was placed in the long half of an 00 gelatin capsule lined with sterile filter paper. The capsule was placed over the starter holes and held in place with a ring of "Dux-seal,"* an asbestos-based plastic sealing compound (Figure 2). One hundred individuals of each sex of the three ips species were introduced in such manner. The infested bolts were placed in sterile 12-pound Kraft paper bags and held for 10 days at 30° C. Bolts treated in the same manner, with the exception of introduction of beetles, served as checks. Following the 10-day holding period, the bolts were dissected and examined for the presence of C. ips. Galleries from which the fungus was isolated were considered positive.

Rearing ips Species In Laboratory Colonies

For the most part, it was not necessary to maintain laboratory colonies of the three ips species. Natural infestations in all stages of development were usually accessible throughout the study

*Johns-Mansville Corporation, Chicago, Illinois.

Figure 2. Gelatin capsule used to hold bark beetles prior to entrance into pine bolt. Capsule held in place with "Duxseal."

period. When beetles were reared in the laboratory, a modification of the techniques described by Clark and Osgood (1964a, b) and Hopping (1961) was used. Slash or loblolly pines were used as the host material. Felled trees were cut into bolts 12 inches long, transferred to the laboratory, and cured for 5 days. The ends of the bolts were dipped in molten paraffin, after curing, and placed in 25- or 50-pound lard cans with two glass emergence jars mounted on ventilation screens on opposing sides of the cans. From 2 to 4 bolts, depending upon their diameter, were placed in each can. From 10 to 20 males of a given species were introduced into the can, and 20 to 40 females were added 1 to 2 days later. The cans were held at 30° C. Reared beetles were either collected in the emergence jars or dissected from the bolts 20 to 25 days after introduction of the females. The beetles were held until needed at 7° C. in petri dishes lined with moist filter paper and containing fresh phloem.

Rearing Ips Species Free of C. Ips

Several methods were tested as a means of obtaining adult ips bark beetles free of C. ips. Surface sterilization of field collected pupae was unsatisfactory. A 5-minute bath in the previously mentioned sterilizing solution proved lethal to most of the pupae, and isolations showed that some of the pupae were internally infected with the fungus. Surface sterilization of adults followed by a 10-day feeding period on macerated phloem containing sorbic acid, methyl parahydroxybenzoate, and streptomycin sulfate

failed to rid the beetles of the fungus. Some beetles, free of C. ips, were reared from surface sterilized eggs utilizing the "phloem sandwich" technique described by Holst (1937). Larval survival, however, was less than 5 per cent and all of the plates became contaminated with mold and bacteria. Use of larval rearing media provided a means of obtaining beetles free of C. ips and other contaminants. Among 18 experimental media tested, the phloem-based medium listed in Table I proved to be the most satisfactory for larval development and was used to obtain adults for brood development and fecundity studies.

Slash pine phloem was used as the phloem source in the diet. The phloem was thoroughly macerated in a blender containing the prescribed amount of water to be used in preparing the diet. The phloem was separated from the liquid by filtration through a double thickness of cheesecloth, and the water-phloem filtrate was used in preparation of the medium. Procedures for preparing the medium were as follows: (1) the agar was placed in the water-phloem filtrate and heated until dissolved; (2) the agar solution was poured into a blender and the combined dry ingredients (including macerated phloem) were slowly added and the whole homogenized; (3) the hot medium was poured from the blender into 20 X 90 millimeter petri dishes at approximately 40 milliliters per dish; (4) a disc of blotting paper the same diameter as the dish was pressed to the surface of the medium and the medium allowed to cool. The acidity of the medium was approximately pH5.

Table 1. Phloem-based, semi-artificial rearing medium for lps bark beetles. Amounts sufficient to make 100 grams. From Yearian and Wilkinson (1965).

Constituent	Amount
	(Grams)
Pine phloem (fresh weight)	46.00
Agar	2.00
Sucrose	1.00
Fructose	1.00
Vitamin Diet Fortification Mixture (In dextrose)*	0.50
Brewer's Yeast	1.00
Soybean protein	1.00
Wesson's salts	0.10
Sorbic acid	0.20
Methyl parahydroxybenzoate	0.15
Streptomycin sulfate	0.01
	(Milliliters)
Water	47.00

*Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

Eggs of the three lps species were obtained from infested host material. The eggs were teased from the oviposition niches with a flattened dissecting needle and transferred to a petri dish with a sable brush. The eggs were surface-sterilized, as previously described, and placed in a petri dish lined with sterile, moist filter paper to incubate at 30° C. Ten newly-hatched larvae were aseptically transferred to a rearing dish. Each larva was inserted into a slit cut in the paper covering. The slit was then closed and sealed with excess agar coated on the paper surface. The dishes were held at 30° C. and 40 to 50 per cent relative humidity.

When the adults were approximately 7 days old, they were removed from the medium in a sterile isolation chamber and transferred to dishes containing fresh medium, according to sex and species. The beetles were held at 70° C. until needed.

Isolations from beetles reared as described above showed them to be free of fungal, bacterial and yeast contaminants.

Observations were made on the duration of the larval and pupal stages of the lps species reared on the phloem-based medium. Five larvae were implanted per dish. The dishes were examined daily and the stage of development recorded. Head capsule width was used as the criterion for differentiating the larval instars (Wilkinson, 1963). When a larva was not visible through the bottom of the dish, it was dissected from the medium, examined, and transferred to a new dish. Pupal weight and survival were also recorded.

Mass Attraction

The attractiveness of pine bolts artificially infested with wild lps calligraphus and lps grandicollis males to both sexes of the same species was demonstrated by Wilkinson (1964). A test was conducted to compare the attractiveness of bolts infested with males freed of C. lps with bolts infested with wild males. Slash pine bolts, 12 inches long and 6 to 8 inches in diameter were cut and cured as previously described. Following curing, the ends of the bolts were dipped in molten paraffin. Six bolts each were infested with two wild lps calligraphus males, introduced 4 and 6 inches, respectively, from the top of the bolts. Six bolts were similarly infested with two lps calligraphus males reared on the phloem-based rearing medium.

After a 24-hour holding period, the bolts were exposed for 24 hours on a constantly rotating turntable (Figure 3) located in a pine woods on the University of Florida Agronomy Farm. The 46-inch diameter turntable was mounted on an "H" frame support and suspended 6 feet above ground level. A 120-volt generator served as the power source for a 1/4 h.p. electric motor coupled to the turntable. A series of gears reduced the turntable speed to 24 revolutions per hour. The two types of bolts were alternately suspended around the edge of the turntable, as shown in Figure 3.

At the end of the exposure period, the bolts were dissected and the number of males, females, and new attacks present were recorded.

Ips Development In C. Ips Stained Bolts

A study was conducted to ascertain (1) whether the three ips species would attack, construct egg galleries, and oviposit in bolts completely stained with C. ips; and if oviposition occurred, (2) whether broods developed normally. Bolts 6 inches long were cut from felled slash pine trees and cured for 3 days. Following curing, holes 0.25 inches in diameter and 3 inches deep were drilled longitudinally in the xylem of both ends of the bolts. The holes were spaced 0.5 inches apart in a circular pattern and 0.5 inches from the outer edge of the phloem. The bolts were inoculated with a mixture prepared by blending ten petri dish cultures of C. ips with 500 milliliters of water. The holes in the bolts were filled with the liquid mixture (using a water dropper) and plugged with 00 corks. The ends of the

Figure 3. Rotating turntable used to expose male infested pine bolts in attraction studies.

bolts were painted with an asphalt pruning compound* to reduce dessication. Bolts treated in the same manner, but inoculated with sterile liquid, served as a check. Sample C. ips inoculated bolts were dissected at 2-day intervals to detect movement of the fungus into the phloem and after 8 days an estimated 95 per cent of the phloem was stained.

Two beetle "starter holes" were drilled in the bark 0.5 inches from one end and on opposing sides of each bolt. Each hole in five stained and unstained bolts was infested with one male and two females of the three ips species. After 15 days, the bolts were dissected and observations made.

Brood Development and Fecundity

Slash pine bolts were used exclusively for brood development and fecundity studies. The bolts, 12 inches long and 3 to 8 inches in diameter, were selectively cut so as to be free of limbs and scars. The bolts were surface decontaminated in the following manner: (1) immediately after cutting, loose bark scales were removed from the bolts, and the ends of the bolts were beveled and dipped for 30 seconds in 70 per cent alcohol containing 200 p.p.m. mercuric chloride; (2) the bolts were placed in an open rack and cured for 5 days at 30° C. and 30 to 40 per cent relative humidity; (3) the ends of the bolts were painted with pruning paint; (4) the bolts were stored in an insect-tight room held at 21° C. until needed; and (5) immediately

*"Tree Kote." Walter E. Clark and Son. Orange, Connecticut.

prior to use, the bolts were sprayed, to the point of run-off, with 70 per cent ethyl alcohol containing the fungistatic agents, methyl parahydroxybenzoate (1.0 per cent) and sorbic acid (1.5 per cent).

Comparison of brood development and fecundity was made between three experimental types of beetles within each Ips species occurring in Florida. The types were as follows:

- (1) Blue stain-free beetles (BSF): Beetles derived from surface sterilized eggs and reared through the larval, pupal, and callow adult stages on the phloem-based medium (Table 1), thus free of C. ips.
- (2) Blue stain beetles (BS): Beetles similarly reared on the phloem-based medium, but inoculated with C. ips by placing them in petri dishes containing sporulating cultures of the fungus for 24 hours.
- (3) Wild beetles (W): Beetles collected in the field from naturally infested trees, logs, or slash.

In order to obtain beetles reared on similar host material and approximately the same age, beetles used in the studies were the F_1 progeny of BSF, BS, and W beetles reared in surface decontaminated slash pine bolts. In general, bolts 3 to 4 inches in diameter were infested with Ips avulsus, bolts 4 to 6 inches in diameter were infested with Ips grandicollis and bolts 6 to 8 inches in diameter were infested with Ips calligraphus. "Starter holes" were drilled in the bark as previously described. Four holes were located mid-way along the length of the bolts and equidistant from each other

on the circumference. A male was introduced into an 00 gelatin capsule lined with sterile filter paper, and a capsule was placed over each of the holes and attached to the bark with "Duxseal." After the male was established for 24 hours, two females were introduced into each capsule. To reduce the possibility of contamination of the bolts through the "starter holes," the above procedures were carried out in an isolation chamber. Each infested bolt was placed in a sterile 25-pound Kraft paper bag. The top of the bag was folded several times and stapled. The bolts were held at 30° C. and 40 to 50 per cent relative humidity.

The bolts were dissected either 20 days (*Ips avulsus*) or 25 days (*Ips calligraphus* and *Ips grandicollis*) following introduction of the females. The teneral adults were removed, sexed, and placed in petri dishes lined with moist, sterile filter paper. Isolations were made from BSF and BS beetles and wood as a check against accidental introduction of contaminants.

In brood development studies, two males, followed by two females per male, were introduced into surface sterilized bolts as described above. The "starter holes" were drilled midway along the length on opposing sides of the bolts (Figure 4). Lots of 20 bolts each were infested with BSF, BS, or W beetles of the three *Ips* species. Two additional lots were infested either with BSF or W *Ips grandicollis* adults. At 5-day intervals (up to 20 days) after introduction of the females, five bolts were selected at random from each of the treatments and dissected. The length of egg galleries and number of egg niches, eggs, larvae, pupae and adults were recorded. Pupal weight was also recorded. Observations were made on *C. Ips* development in BS and W bolts, and isolations were made from BSF, BS, and W bolts.

Figure 4. Pine bolt rearing unit used in brood development studies.

Bolts used in fecundity studies were prepared as those for brood development. Only one female, however, was introduced with each male. Lots of ten bolts each were infested with each of the three experimental types of each lps species. The bolts were dissected 10 days after introduction of the females. The females were removed and re-introduced into fresh bolts with newly established males. Observations were carried out over a 30-day period. At each dissection, the length of egg gallery, number of egg niches, and number of eggs were recorded for each female.

RESULTS AND DISCUSSION

Fungus Isolations

Ceratocystis ips (Rumbold) C. Moreau was the only member of the genus found associated with ips avulsus, ips calligraphus and ips grandicollis in Florida. A perithecium and spores of C. ips are shown in Figures 5 and 6, respectively. Another fungus, probably Tuberculariella ips Leach, Orr, and Christensen, was commonly found in association with ips avulsus. In addition, several undetermined fungi, yeasts, and bacteria were frequently isolated from the wood and galleries of trees attacked by the three beetle species.

Ceratocystis ips was easily isolated from the wood and phloem of beetle infested trees. Perithecia of the fungus were abundant in old egg galleries of all three ips species. The fruiting structures were commonly found in uningested phloem adjacent to the egg galleries shortly before pupation by the larvae. Isolations from frass plugging the egg niches and loose in the egg galleries consistently yielded C. ips. Isolations from 100 frass plugs from egg niches of ips avulsus, ips calligraphus and ips grandicollis showed 23, 100, and 97 of them, respectively, contained the fungus. Perithecia were frequently observed in the frass plugs shortly after the eggs hatched.

Figure 5. Perithecium of Ceratocystis ips. Approx. 75X.

Figure 6. Ascospores of Ceratocystis ips. Approx. 2000X.

Isolations from each of the life stages of Ips avulsus, Ips calligraphus, and Ips grandicollis showed the fungus to be primarily associated with the late larval and adult stages (Table 2). Eggs and first-stage larvae of the three beetle species were internally free of the fungus, but C. ips was isolated from surface-sterilized second and third instar larvae. Ips avulsus larvae showed the highest incidence of internal contamination in both the second and third instars, 74 and 100 per cent, respectively. Surface-sterilized Ips avulsus pupae were found to be free of C. ips, but isolations from 100 Ips calligraphus and Ips grandicollis pupae yielded 23 and 4 isolates, respectively. Surface-sterilized teneral adults showed a high incidence of C. ips isolation. No difference was observed in the degree of internal contamination of males and females of Ips calligraphus and Ips grandicollis, but 35 per cent of the Ips avulsus males contained the fungus while only 8 per cent of the females were internally contaminated. C. ips was isolated from every non-sterilized Ips calligraphus and Ips grandicollis adult cultured. Only Ips avulsus with 85 per cent of the males and 83 per cent of the females showed less than 100 per cent incidence of contamination.

As previously mentioned, Ips avulsus larvae showed the highest incidence of internal contamination. This can possibly be explained by the larval habits of this species. The larvae were usually confined to within 1 centimeter of the egg gallery. They tunneled more or less obliquely to the egg gallery and formed a "fan-shaped" gallery. The phloem surrounding the larvae soon became stained with C. ips after the eggs hatched, and as the larvae enlarged the gallery

Table 2. Relative frequency of Ceratocystis lps Isolation from lps avulsus, lps calligraphus, and lps grandicollis.

Stage of Development	<u>lps</u> <u>avulsus</u>		<u>lps</u> <u>calligraphus</u>		<u>lps</u> <u>grandicollis</u>	
	Number	%	Number	%	Number	%
SURFACE-STERILIZED:						
Egg	100	0	100	0	100	0
Larva						
1st Instar	100	0	100	0	100	0
2nd Instar	100	74	100	16	100	33
3rd Instar	100	100	100	84	100	80
Pupa	100	0	100	23	100	4
Teneral adult						
male	100	35	100	60	100	89
female	100	8	100	55	100	83
NON-STERILIZED:						
Teneral adult						
male	100	85	100	100	100	100
female	100	83	100	100	100	100

and fed, stained phloem was consumed. lps calligraphus and lps grandicollis larvae, on the other hand, generally mined at a right angle to the egg gallery. The larval galleries extended several centimeters from the egg gallery and were not greatly enlarged.

C. lps growth did not overtake the larvae until the late third instar or until after pupation. Some lps calligraphus and lps grandicollis larvae, however, mined more or less parallel to the egg gallery and consumed stained phloem, thus accounting for a number of the larvae from which the fungus was isolated.

The data (Table 2) indicating lps calligraphus and lps grandicollis pupae were internally infected with C. lps appear questionable. Previous workers (Leach et al., 1934, and Grosmann, 1930) failed to isolate Ceratocystis species from surface-sterilized lps species pupae. In the present study all third instar lps avulsus larvae examined were found to be infected with C. lps, yet none of the pupae of this species yielded the fungus. Incomplete surface sterilization of the lps calligraphus and lps grandicollis pupae cultured would explain the results obtained since the pupal chambers of both species frequently contained fruiting structures of the fungus and the pupae were doubtlessly externally contaminated with C. lps spores. Grosmann (1930), however, found viable yeast cells in the lps typographus pupae; therefore, the possible validity of the above data cannot be totally discounted.

Frequency of C. lps Transmission

Both male and female lps avulsus, lps calligraphus and lps grandicollis adults were found capable of transmitting Ceratocystis lps (Table 3). Males of all three species, that successfully attacked the bolts, were also successful in transmitting the fungus. Similar observations were made with lps calligraphus and lps grandicollis females, but lps avulsus females showed less than 100 per cent transmission. Of 92 successful attacks by lps avulsus females, two attacks were found that showed no sign of blue staining and from which C. lps was not isolated. Since the males of this species

Table 3. Relative frequency of Ceratocystis ips transmission by ips avulsus, ips calligraphus, and ips grandicollis.

Beetle Species	Successful Attacks			Unsuccessful Attacks		
	<u>C. ips</u> Present	<u>C. ips</u> Absent	<u>C. ips</u> Trans- mission	<u>C. ips</u> Present	<u>C. ips</u> Absent	<u>C. ips</u> Trans- mission
<u>ips avulsus</u>						
Male	83	0	100.0	8	9	47.1
Female	90	2	97.8	5	3	62.5
<u>ips calligraphus</u>						
Male	100	0	100.0	0	0	0.0
Female	95	0	100.0	5	0	100.0
<u>ips grandicollis</u>						
Male	97	0	100.0	3	0	100.0
Female	93	0	100.0	7	0	100.0
Check	0	0	0.0			

demonstrated 100 per cent transmission, the probability of an ips avulsus gallery system being free of C. ips appeared to be unlikely.

Even though some attacks by the beetles were unsuccessful, C. ips infection frequently occurred (Table 3). An attack was considered unsuccessful when a male failed to construct a nuptial chamber and a female failed to excavate at least a centimeter of gallery. All unsuccessful attacks by ips calligraphus and ips grandicollis adults were stained by the fungus. Of 17 unsuccessful attacks by ips avulsus males, C. ips was isolated from 8, and 5 of 8 unsuccessful attacks by females yielded C. ips cultures. Even though the attacks were

considered unsuccessful, most of the beetles fed for a brief period, and contacted the phloem and xylem.

Upon dissection, wood of successfully attacked bolts was visibly stained by C. ips. The stain was confined to the immediate vicinity of the attack and did not extend deeply into unscored areas of the phloem. Perithecia were beginning to form in the nuptial chamber made by the males and in the basal portion of the galleries constructed by the females. Stain was not visible in the wood or phloem of bolts where attacks were not successful unless the beetle had chewed through the phloem to the wood. In any case, staining was very slight, and isolations were necessary to detect the fungus.

Larval and Pupal Development on Phloem-Based Rearing Medium

Ips avulsus, Ips calligraphus, and Ips grandicollis larval and pupal developmental rates on the phloem-based rearing medium were similar at 30° C. (Table 4). The mean length of the larval period was 8.4 days for Ips avulsus, 8.9 days for Ips calligraphus and 9.2 days for Ips grandicollis. With the exception of Ips avulsus, the mean developmental time for the three larval instars progressively increased. Second instar Ips avulsus larvae required an average of 3.2 days to complete development while the third instar required 2.8 days. Although no data were recorded, the pre-pupal stage for the three species was approximately one day. The pupal period for Ips avulsus, Ips calligraphus and Ips grandicollis averaged 2.8, 3.7, and 3.6 days, respectively.

Table 4. Mean larval and pupal development of Ips avulsus, Ips calligraphus, and Ips grandicollis on phloem-based, semi-artificial rearing medium at 30° C.

Stage of Development	<u>Ips avulsus</u>		<u>Ips calligraphus</u>		<u>Ips grandicollis</u>	
	Mean	No. Ob- (days) served	Mean	No. Ob- (days) served	Mean	No. Ob- (days) served
Larva (total)	8.4±0.51	11	8.9±0.27	45	9.2±0.20	51
1st Instar	2.5±0.22	15	2.7±0.13	86	2.6±0.10	87
2nd Instar	3.2±0.29	15	3.0±0.10	64	2.8±0.12	81
3rd Instar	2.8±0.30	13	3.7±0.22	49	3.6±0.18	71
Pupa	2.8±0.36	9	3.3±0.10	45	3.0±0.18	43

The incubation period of newly deposited surface-sterilized eggs was found to average 2.3, 2.7, and 2.8 days for Ips avulsus, and Ips calligraphus, and Ips grandicollis, respectively. With the mean incubation period added to the mean larval and pupal developmental times, the average time required from egg to callow adult was 13.5 days for Ips avulsus, was 14.9 days for Ips calligraphus, and was 15.0 days for Ips grandicollis. After 7 days, teneral adults reared on the medium were capable of infesting pine bolts and establishing broods. Thus, allowing a day for attack, mating and initiation of oviposition, a complete life cycle, from egg to egg, averaged 21.5, 22.9, and 23.0 days for Ips avulsus, Ips calligraphus, and Ips grandicollis, respectively. These developmental rates are comparable to generalized life cycles for the three species given by Thatcher (1960).

Larval mortality on the medium was high. Of 100 newly hatched larvae implanted at the beginning of the study, only 13 lps avulsus, 50 lps calligraphus, and 49 lps grandicollis larvae reached the pupal stage. Most of the mortality was attributed to handling since it was necessary to remove many of the larvae from the medium daily to determine the stage of development. Additional observations on several hundred larvae, handled only when implanted in the medium, showed larval survival to be 56.7 per cent for lps avulsus, 80.6 per cent for lps calligraphus, and 68.3 per cent for lps grandicollis.

lps avulsus pupae reared on the medium weighed significantly less ($p=.05$) than wild (W) pupae; 1.66 and 2.20 milligrams, respectively. Suggested reasons for this difference are discussed later. lps calligraphus and lps grandicollis pupae reared on the medium averaged 10.77 and 5.17 milligrams and were significantly larger ($p=.05$) than wild (W) pupae (see Table II). The increased weight of medium reared lps calligraphus and lps grandicollis pupae was attributed to two factors. First, the medium contained carbohydrates, proteins, vitamins, and minerals in addition to phloem (see Table I) and was no doubt richer in available nutrients than natural food. Secondly, the pupae were reared from surface-sterilized eggs, and the larvae were free of internal parasites, particularly nematodes. Massey (1957, 1960, 1962) and Nickle (1963a, b) found a high incidence of nematode infection in certain species of bark beetles and indicated the nematodes reduced the vigor of the beetles. Observations incidental to this study, showed lps calligraphus and lps grandicollis larvae, pupae, and adults were frequently infected with a number of

nematodes. The removal of these parasites by rearing the beetles from surface-sterilized eggs, could account for the significant increase in weight. Beetles reared on the medium and transferred to surface decontaminated pine bolts also produced pupae significantly larger than wild (W) pupae (Table II).

Mass Attraction

A comparison of the attractiveness of wild (W) and blue stain-free (BSF) Ips calligraphus male infested bolts is presented in Table 5. Although BSF bolts attracted more females, Chi-square analysis of the data failed to show a significant difference in the relative attractiveness of the two types of bolts. While one observation is usually not sufficient to draw a conclusion, the conclusiveness of the attraction test clearly indicated that C. ips was not a factor in Ips calligraphus mass attraction.

Person (1931) suggested that the odor caused by yeast fermentation influenced mass attraction of bark beetles. Subsequent work (Anderson, 1948; Vité' and Gara, 1961, 1962; Wood and Vité', 1961; and Wood, 1962) established that live male Ips bark beetles in freshly attacked host material created the attraction. Wood and Vité' (1961) noted that for yeasts and/or other microorganisms to be responsible for the attraction they would have to be specifically associated with the male beetles. No such association has been reported in the literature. Vité' and Gara (1962) treated logs with materials toxic to yeasts, but the logs were still attractive to other beetles when attacked by the pioneering male bark beetles. Anderson (1948) innoculated pine

Table 5. Attractiveness of W and BSF ips calligraphus male infested slash pine bolts.

	Number of Beetles Recovered			New Attacks
	Females	Males*	Total	
BSF Bolts				
1	3	4	7	3
2	9	5	14	3
3	2	1	3	0
4	5	3	8	1
5	7	3	10	1
6	7	3	10	1
Total	33	19	52	9
W Bolts				
1	10	6	16	4
2	0	1	1	0
3	4	2	6	1
4	3	4	7	2
5	4	2	6	0
6	6	3	9	1
Total	27	18	45	8

*Includes males introduced in laboratory.

logs with yeast isolated from ips pini galleries, and found they were not more attractive than the check bolts. Although the test presented herein (Table 5) was designed to ascertain the effect of C. ips on attraction, BSF beetles used in the test were also free of yeasts. Since bolts infested with males freed of yeasts and fungi attracted more beetles than bolts with males contaminated with the micro-organisms, the assumption that yeasts are not a factor in mass attraction was again corroborated.

Ips Development In C. Ips Stained Bolts

Attempts to establish ips avulsus, ips calligraphus and ips grandicollis broods in bolts inoculated with C. ips 8 days prior to introduction of the beetles were unsuccessful. The males of all three species constructed nuptial chambers, and females tunneled in the stained phloem but, with two exceptions, deposited no eggs. An ips avulsus female laid two eggs shortly after initiating the egg gallery. The eggs hatched and at dissection, 15 days, the larvae had pupated. The pupae weighed 2.05 and 2.14 milligrams, respectively, and were within the range of pupal weights given in Table II. An ips calligraphus female laid one egg and the resultant pupa was recovered at dissection. In handling, the pupa was crushed before it could be weighed, but it appeared comparable in size to laboratory reared ips calligraphus pupae. The remainder of the females of all three beetle species abandoned the bolts without ovipositing although some constructed galleries the entire length of the bolts.

Females of the three ips species produced apparently normal broods in the check bolts, thus it appeared that C. ips rendered the infected bolts unsuitable as breeding material for the beetles. The infected bolts appeared notably drier than the check bolts. Anderson (1948) induced ips pinl attack in logs dried for a year. He noted that the females constructed egg galleries, but no brood was produced; a situation very similar to that previously described for the stained bolts. Mathiesen-Kærlik (1960) found that certain insect associated Ceratocystis species utilized several amino acids, sugars, and vitamins and Mathre (1964c) demonstrated that C. ips significantly

reduced the fructose content of ponderosa pine sapwood. Whether a similar alteration of the chemical composition of the xylem and phloem was sufficient to render the bolts unsuitable cannot be answered here.

Even though the presence of C. ips in the bolts apparently created conditions not conducive to oviposition by the beetles, there was no evidence that the fungus rendered the phloem unsuitable as larval food. Although only three eggs were deposited, the resultant larvae developed to the pupal stage. The developmental time and size of the pupae did not appear to be retarded, although White (1962) has shown that blue stain fungi reduce the nutritional value of Scot's pine (Pinus sylvestris L.) sapwood as food for the larvae of Hylotrupes bajulus L. Additional observations are necessary to ascertain the effect C. ips may have on the nutritional value of pine phloem as food for ips bark beetles.

Effect of C. ips on Brood Development and Fecundity

Egg gallery construction

In brood development test bolts seeded with two males and two females per male, the females usually excavated their egg galleries in opposite directions; one extending upward and the other downward from the nuptial chamber. Occasionally both females cut their egg galleries side by side and the galleries were separated only by a narrow strip of phloem (usually less than 0.5 centimeter). In general egg gallery excavation ceased when the female reached the end of the test bolt. Some females continued to burrow around the

end of the bolt and eventually turned back parallel with the grain of the wood forming a "U" shaped gallery. Some Ips grandicollis and Ips calligraphus females burrowed out the end of the bolt and re-entered at another site on the bolt.

Egg gallery construction and oviposition by all three species were completed 10 days after introduction of the females, and most of the activity took place the first 5 days. Statistical analysis of gallery length at 5, 10, 15, and 20 days failed to show a significant difference between mean lengths at any of the dissection dates. Thus, all gallery length measurements, regardless of dissection date, were used to determine mean gallery lengths for the W, BSF, and BS beetles of each species. As shown in Table 6, W Ips avulsus females constructed significantly ($p=.05$) longer galleries than BSF and BS females, while BSF Ips calligraphus and Ips grandicollis females constructed significantly ($p=.05$) longer galleries than W and BS females.

The eggs were deposited at irregular intervals in niches cut in both sides of the gallery. When either Ips calligraphus or Ips grandicollis females constructed galleries side by side, most of the eggs were deposited in the outer wall of their respective galleries, and very few eggs were deposited in the narrow phloem strip separating the galleries. Ips avulsus females also deposited more eggs in the outer wall, when the galleries were side by side, but several eggs were deposited in the adjacent gallery walls. Ips avulsus females, particularly BSF and BS females, spaced their eggs farther apart in the egg galleries than did the other two species (Table 6). Ips grandicollis eggs were more closely spaced than Ips calligraphus eggs.

Table 6. Mean egg gallery length and spacing between eggs of W, BSF, and BS ips avulsus, ips calligraphus, and ips grandicollis females in slash pine bolts at 30°C.

Species	Number of Galleries	Mean Length (cm.)	Spacing Between Eggs (cm.)
<u>ips avulsus</u>			
W	46	13.5±1.4*	0.59
BSF	37	11.6±1.3	0.68
BS	30	12.2±1.1	0.86
<u>ips calligraphus</u>			
W	40	14.2±2.2	0.55
BSF	36	20.1±1.8*	0.48
BS	37	14.9±1.8	0.49
<u>ips grandicollis</u>			
W	73	11.5±1.7	0.39
BSF	61	13.9±2.2*	0.34
BS	33	11.9±1.8	0.31

*Significantly different ($p=.05$) from others within same species. Duncan's New Multiple Range Test.

Although egg gallery excavation and oviposition were terminated after approximately 10 days, the females remained in the galleries. Most of the ips avulsus females abandoned their galleries between the 15- and 20-day dissection dates, but some remained until the 20-day dissection. ips grandicollis and ips calligraphus females remained in the gallery for the entire 20-day observation period. Approximately 10 days after introduction of the females, the males of all three species vacated the nuptial chamber and re-attacked the bolts.

Ceratocystis ips in brood development test bolts

Ceratocystis ips was present in each W and BS bolt examined, but careful examination of BSF bolts showed them to be free of the fungus. Staining of the sapwood of BSF bolts was observed, but the stain did not penetrate the sapwood as deeply as C. ips and was somewhat darker in color. Since isolations from discolored sapwood did not yield microbial growth and microscopic examination did not reveal fungus hyphae or fruiting structures, it was assumed that the stain was chemical in nature. An occasional BSF bolt became contaminated with mold, and such bolts were discarded and the data were not included in the study.

Growth of C. ips through the gallery systems of all three ips species was somewhat faster in the BS bolts than the W bolts, but the developmental pattern was similar to that described by Leach et al. (1934). Staining was not visible in W bolts after 5 days, but the fungus was easily isolated from all sections of nuptial chambers and egg galleries of the three beetle species. In most BS bolts slight staining was visible in the nuptial chamber and basal portion of the egg gallery. In a few bolts perithecia were present in frass plugging the first egg niches. After 10 days the nuptial chambers and basal portion of the egg galleries in both W and BS bolts were heavily stained and bore scattered perithecia. The fungus had begun to spread perpendicularly to the egg galleries through the larval tunnels, and the older members of ips avulsus broods were overtaken by C. ips. Fifteen days after introduction of

the females the entire length of the egg galleries was stained and lined with perithecia. Figure 7 shows C. lps perithecia lining a section of a 15-day old BS lps grandicollis gallery 5 centimeters from the nuptial chamber. Figure 8 shows a comparable section of BSF lps grandicollis gallery. At 15 days the entire gallery system of lps avulsus broods, and most of the gallery system of lps calligraphus and lps grandicollis broods were covered by the fungus. Perithecia and coremia were beginning to form in the older larval tunnels, pupal chambers, and unconsumed areas of phloem. By 20 days the fungus had grown over the entire gallery system of all three lps species.

Brood size and mortality

Mean brood size (average number of eggs, larvae, pupae, and/or adults per egg gallery) was determined using all observations since statistical analysis failed to reveal any significant differences in size of the broods at the different dissection dates. Wild (W) lps avulsus females produced significantly ($p=.05$) larger broods than BSF and BS females (Table 7). Blue stain-free (BSF) lps calligraphus broods were significantly larger than W and BS broods. Both BSF and BS lps grandicollis broods contained significantly ($p=.05$) more individuals than W broods.

Brood mortality was based upon the difference between the number of eggs niches cut by the females and the number of offspring recovered at dissection of the bolts. As shown in Table 7, mortality in all three species was highest in the W bolts, and overall mortality

Figure 7. Section of 15-day old BS Ips grandicollis gallery 5 centimeters from nuptial chamber. Note perithecia of Ceratocystis ips lining gallery and masses of ascospores at tips of perithecia. Approx. 40X.

Figure 8. Section of 15-day old BSF Ips grandicollis gallery 5 centimeters from nuptial chamber. Note absence of Ceratocystis ips. Approx. 10X.

Table 7. Mean size and mortality of W, BSF, and BS lps avulsus, lps calligraphus, and lps grandicollis broods in slash pine bolts at 30° C.

Species	Number of Broods Observed	Brood Size (No. Offspring)	Brood Mortality (%)
<u>lps avulsus</u>			
W	46	20.7±1.6	5.1
BSF	37	16.4±1.7	3.2
BS	30	14.0±2.0	2.7
<u>lps calligraphus</u>			
W	40	28.5±3.8	16.1
BSF	36	35.7±3.9*	13.5
BS	37	31.8±3.9	8.6
<u>lps grandicollis</u>			
W	73	28.5±2.6	9.4
BSF	61	39.9±2.9*	4.6
BS	33	39.6±3.9*	6.5

*Significantly larger ($p=.05$) from others within same species.
Duncan's New Multiple Range Test.

was greatest in lps calligraphus broods. The increased mortality in the W broods was attributed, in part, to parasites. A parasitic mite was observed attacking the eggs of all three beetle species, and was more common in lps calligraphus bolts. As previously mentioned, the beetles in all stages, with the exception of the egg, were infected with nematode parasites. The possible pathogenic effect of these nematodes has not been demonstrated, however. A fungus was also found infecting the eggs of the three beetle species.

Brood composition

The composition of W, BSF, and BS lps avulsus, lps calligraphus, and lps grandicollis broods at the different dissection dates is depicted in Figures 9, 10, and 11, respectively. The data are also numerically presented in Table 8. As mentioned in the previous section, there were significant differences in the size of W, BSF, and BS broods within each beetle species. Such differences made direct comparison of brood composition difficult. To obtain a clear picture of the make-up of the broods and the shift in composition of the broods with time, absolute brood composition data were converted to relative values (Table 9). The relative composition of W, BSF, and BS broods within species were compared at each dissection date using Chi-square analysis (Table 10) to detect differences in rate of development.

Although W lps avulsus broods were significantly larger than BSF and BS broods, the relative make-up of the three types of broods was not significantly different after 5 days. At 10 and 15 days, however, W lps avulsus broods appeared to have developed faster, and the relative composition of W broods was significantly different from BSF and BS broods (Table 10). Wild (W) broods contained proportionally fewer eggs and first instar larvae and more pupae at 10 days (Table 9). After 15 days W broods were predominately pupae and callow and teneral adults while BSF and BS broods were composed of proportionally more second and third instar larvae and pupae. At 15 days, none of the W lps avulsus broods examined contained second instar larvae. By the final dissection date (20 days) all three lps avulsus broods were

Figure 9. Mean composition of *W*, *BSF*, and *BS lps avulsus* broods in slash pine bolts at 5, 10, 15, and 20 days at 30° C.

Figure 10. Mean composition of W, BSF, and BS *Ips calligraphus* broods in slash pine bolts at 5, 10, 15, and 20 days at 30° C.

Figure 11. Mean composition of W, BSF, and BS *Ips grandicollis* broods in slash pine bolts at 5, 10, 15, and 20 days at 30° C.

Table 8. Mean composition of W, BSF, and BS lps avulsus, lps calligraphus, and lps grandicollis broods in slash pine bolts at 5, 10, 15, and 20 days at 30° C.

Species	Time (days)	Stage of Development					
		Egg	Larval Instar			Pupa	Adult
			1	2	3		
<u>Ips avulsus</u> <u>W</u>	5	9.8±1.6	9.8±0.8	3.2±0.8	---	---	---
	10	0.8±0.1	0.8±0.1	7.3±0.9	13.2±3.5	2.2±2.2	---
	15	---	---	---	1.4±1.1	2.7±1.7	11.6±3.7
	20	---	---	---	---	---	20.0±5.1
BSF	5	4.6±0.8	3.1±0.4	3.6±0.8	---	---	---
	10	2.0±1.8	1.7±1.4	4.4±1.1	7.5±4.8	0.3±0.1	---
	15	---	---	3.8±2.9	11.8±6.1	2.4±1.8	3.2±2.9
	20	---	---	---	---	---	19.1±4.9
BS	5	4.3±1.5	2.8±1.6	1.3±1.2	---	---	---
	10	1.0±0.2	3.0±1.8	5.3±2.7	6.5±2.9	0.3±0.1	---
	15	---	---	2.1±1.9	4.9±2.1	3.1±2.5	3.3±2.7
	20	---	---	---	---	---	18.0±4.8
<u>Ips calligraphus</u> <u>W</u>	5	10.3±6.3	4.7±3.2	1.7±1.6	---	---	---
	10	5.4±2.7	4.5±2.7	7.9±4.8	11.7±5.7	---	---
	15	---	---	4.6±1.5	15.2±6.2	5.6±2.6	1.3±0.9
	20	---	---	---	3.8±2.2	3.0±0.6	28.8±10.3

Table 8. Cont.

Species	Time (days)	Stage of Development				
		Egg	Larval Instar		Pupa	Adult
			1	2	3	
<u><i>Ips calligraphus</i></u>						
BSF	5	12.1±6.4	8.3±6.4	3.6±2.9	-- --	-- --
	10	6.8±4.8	7.1±2.7	13.0±4.2	-- --	-- --
	15	-- --	-- --	4.8±4.2	20.3±5.1	4.5±3.7
	20	-- --	-- --	-- --	1.0±0.4	30.5±12.1
BS	5	9.9±3.9	11.6±5.1	7.8±2.8	-- --	-- --
	10	3.0±2.5	3.4±0.8	8.9±3.9	10.2±4.4	-- --
	15	-- --	-- --	4.2±4.1	10.5±5.2	9.3±4.4
	20	-- --	-- --	-- --	2.6±0.3	32.8±6.8
<u><i>Ips grandicollis</i></u>						
W	5	13.1±4.7	11.4±3.3	1.4±0.3	-- --	-- --
	10	4.2±2.1	6.8±2.3	14.6±5.4	6.1±4.5	-- --
	15	-- --	0.5±0.1	3.5±2.1	16.1±1.5	-- --
	20	-- --	-- --	-- --	4.0±2.8	17.4±6.7
BSF	5	18.8±3.5	12.4±2.9	1.6±0.3	-- --	-- --
	10	3.8±3.1	6.3±2.2	14.9±2.5	20.4±3.2	-- --
	15	-- --	2.0±1.6	5.4±3.8	21.3±3.8	5.0±2.6
	20	-- --	-- --	-- --	2.2±2.1	31.5±5.1
BS	5	17.1±5.3	10.6±4.6	2.2±0.4	-- --	-- --
	10	3.8±0.5	8.3±4.0	22.6±7.1	10.8±2.6	-- --
	15	-- --	-- --	4.2±2.3	14.2±5.3	7.2±0.6
	20	-- --	-- --	-- --	1.9±0.1	37.4±6.1

Table 9. Relative composition (percentage) of W, BSF, and BS *Ips avulsus*, *Ips calligraphus*, and *Ips grandicollis* broods in slash pine bolts at 5, 10, 15, and 20 days at 30° C.

Species	Time (days)	Stage of Development					
		Egg	Larval Instar			Pupa	Adult
			1	2	3		
<u>Ips avulsus</u>							
W	5	43.2	42.8	13.9	-- --	-- --	-- --
	10	3.2	3.5	29.9	54.3	9.1	-- --
	15	-- --	-- --	-- --	8.9	17.1	73.8
	20	-- --	-- --	-- --	-- --	-- --	100.0
BSF	5	40.6	27.8	31.6	-- --	-- --	-- --
	10	12.5	11.3	27.2	47.1	1.7	-- --
	15	-- --	-- --	16.0	56.6	11.7	15.5
	20	-- --	-- --	-- --	-- --	-- --	100.0
BS	5	50.0	32.6	17.4	-- --	-- --	-- --
	10	6.1	18.5	32.9	40.2	2.0	-- --
	15	-- --	-- --	15.8	36.4	23.3	24.2
	20	-- --	-- --	-- --	-- --	-- --	100.0
<u>Ips calligraphus</u>							
W	5	61.1	28.1	10.1	-- --	-- --	-- --
	10	18.3	15.2	26.8	39.6	-- --	-- --
	15	-- --	-- --	8.6	62.5	23.2	5.5
	20	-- --	-- --	-- --	10.5	8.4	80.9
BSF	5	50.4	34.7	14.8	-- --	-- --	-- --
	10	15.2	15.7	28.8	40.1	-- --	-- --
	15	-- --	-- --	13.6	57.2	16.4	12.6
	20	-- --	-- --	-- --	3.9	8.6	87.4
BS	5	33.8	39.4	26.7	-- --	-- --	-- --
	10	11.7	13.0	34.9	39.8	-- --	-- --
	15	-- --	-- --	13.6	34.4	21.3	30.6
	20	-- --	-- --	-- --	6.6	7.8	85.6
<u>Ips grandicollis</u>							
W	5	50.5	44.1	5.3	-- --	-- --	-- --
	10	22.3	19.4	41.2	17.1	-- --	-- --
	15	1.5	12.0	54.7	31.6	-- --	-- --
	20	-- --	-- --	-- --	15.3	17.8	66.8

Table 9. Cont.

Species	Time (days)	Stage of Development					
		Egg	Larval Instar			Pupa	Adult
			1	2	3		
<hr/>							
<u>Ips grandicollis</u>							
BSF	5	57.3	37.6	4.9	-- --	-- --	-- --
	10	12.4	13.1	31.3	43.0	-- --	-- --
	15	4.3	11.8	46.7	18.7	18.6	-- --
	20	-- --	-- --	-- --	5.9	8.6	85.4
BS	5	57.2	35.4	7.2	-- --	-- --	-- --
	10	8.2	18.4	49.7	23.6	-- --	-- --
	15	-- --	8.9	30.3	45.2	15.3	-- --
	20	-- --	-- --	-- --	3.3	4.7	9.2

composed of adults. Most of the W adults were late tenerals, and some had begun to emerge from the bolts. Most of the BSF and BS adults were light in color with some still in the callow stage. Emergence from BSF and BS bolts was not observed at 20 days.

A comparison of Ips calligraphus broods failed to show consistent differences in the relative make-up of W, BSF, and BS broods. At 5 days BS broods were composed of a significantly higher percentage of second instar larvae and relatively fewer eggs than W and BSF broods (Tables 9 and 10). But, after 10 days the relative compositions of the three brood types were not significantly different. Again, at 15 days BS broods appeared further developed than W and BSF broods and contained proportionally more adults. At 20 days, however, no significant difference was detected in the relative composition of the three brood types.

Table 10. Chi-square comparison of W, BSF, and BS lps avulsus, lps calligraphus, and lps grandicollis brood composition at 5, 10, 15, and 20 days at 30° C.

Dissection Date	Brood Comparison		
	W vs. BSF	BS vs. BSF	W vs. BS
<u>lps avulsus</u>			
5	NS	NS	NS
10	**	NS	**
15	**	NS	**
20	NS	NS	NS
<u>lps calligraphus</u>			
5	NS	**	**
10	NS	NS	NS
15	NS	**	**
20	NS	NS	NS
<u>lps grandicollis</u>			
5	NS	NS	NS
10	**	**	**
15	**	**	**
20	**	NS	**

NS Not significantly different

** Significantly different ($p=.01$) from others of the same species on same dissection date.

The relative make-up of lps grandicollis broods was not significantly different at 5 days. At the 10-day dissection BSF broods appeared more advanced than the W and BS broods with proportionately more third instar larvae (Table 9). On the basis of relatively fewer eggs and first instar larvae and more second and third instar larvae, BS broods also appeared to be more advanced than W broods. Both at the 15-and 20-day dissections BSF and BS broods differed

significantly (Table 10) from the W broods. After 15 days, BSF and BS broods contained pupae while W broods were composed of eggs and larvae. Blue stain (BS) broods contained no eggs and proportionately more third instar larvae at 15 days and were significantly different from BSF broods. After 20 days W, BSF, and BS broods were composed of third instar larvae, pupae, and adults, but the relative number of adults in BSF and BS broods was significantly greater than the W broods.

Pupal weight

Pupal weight was used as an indicator of the effect of the presence or absence of Ceratocystis ips on the nutritive qualities of the phloem in the test bolts. As shown in Table 11, W ips avulsus pupae were significantly ($p=.05$) heavier than BSF and BS pupae. The average weights of BSF and BS ips avulsus pupae were not significantly different. Both ips calligraphus and ips grandicollis BSF and BS pupae weighed significantly more than W pupae. As with ips avulsus, there were no significant differences between the weight of BSF and BS pupae of ips calligraphus and ips grandicollis.

Sex ratio

The sex ratio of adults recovered from the brood development test bolts is given in Table 12. The ratio of males to females in each species and each type within species appeared to be one to one.

Fecundity

In fecundity tests many eggs hatched before dissection of the

Table 11. Mean weight (mg.) of 100 W, BSF, and BS Ips avulsus, Ips calligraphus, and Ips grandicollis pupae reared in slash pine bolts at 30° C.

Beetle Species	Type of Pupa		
	W	BSF	BS
<u>Ips avulsus</u>	2.20±0.11*	1.71±0.23	1.76±0.11
<u>Ips calligraphus</u>	9.74±0.25	10.28±0.40*	10.17±0.48*
<u>Ips grandicollis</u>	3.71±0.11	4.54±0.14*	4.23±0.16*

* Significantly different ($p=0.5$) from others within same species. Duncan's New Multiple Range Test.

bolts, and actual egg counts were not feasible. Examination of several hundred egg niches before the eggs hatched showed that each niche, without exception, contained an egg. Consequently, the number of egg niches cut per female was considered equal to the number of eggs laid and was recorded as such.

Wild (W) Ips avulsus females constructed significantly ($p=.01$) longer egg galleries and deposited significantly more eggs than BSF and BS females (Table 13). The average number of eggs per centimeter of egg gallery, however, was comparable for the three female types. The number of eggs laid by both BSF and BS Ips calligraphus and Ips grandicollis females was significantly ($p=.01$) greater than that laid by W females. Blue stain-free (BSF) Ips calligraphus females also produced ($p=.05$) more eggs than BS females. The average length of egg gallery cut by the three types of Ips calligraphus and Ips

Table 12. Sex ratio of W, BSF, and BS Ips avulsus, Ips calligraphus, and Ips grandicollis adults reared in slash pine bolts at 30° C.

Species	Number Examined	Number Males	Number Females	Sex Ratio Males:Females
<u>Ips avulsus</u>				
W	92	48	44	1:0.92
BSF	60	32	28	1:0.88
BS	70	37	33	1:0.89
<u>Ips calligraphus</u>				
W	102	52	50	1:0.96
BSF	100	52	48	1:0.92
BS	99	52	47	1:0.90
<u>Ips grandicollis</u>				
W	167	86	81	1:0.94
BSF	132	72	60	1:0.83
BS	115	56	59	1:1.05

grandicollis females was not significantly different, but BSF and BS females of both species laid more eggs per centimeter of gallery than W females.

Discussion of the Effect of C. Ips and Other Organisms on Brood Development and Fecundity

The supposition is often presented that bark beetles and their Ceratocystis associates are capable of independent development, but optimum development of both organisms takes place only when the two occur in conjunction (Dixon and Osgood, 1961). Such seems to be the case with C. Ips, but not necessarily with Ips avulsus, Ips calligraphus and Ips grandicollis. The fungus appears not only to be

Table 13. Mean length of egg gallery, number of eggs laid, and number of eggs deposited per centimeter of gallery by W, BSF, and BS *lps avulsus*, *lps calligraphus*, and *lps grandicollis* females at 30 days in slash pine bolts at 30° C.

	Number Females Observed	Mean Gallery Length (cm.)	Mean No. Eggs Laid	Mean No. Eggs Per cm. Gal- lery
<u><i>lps avulsus</i></u>				
W	10	51.2 $\pm$ 5.75**	77.0 $\pm$ 13.8*	1.5
BSF	11	31.2 $\pm$ 6.83	54.0 $\pm$ 6.8	1.7
BS	11	36.8 $\pm$ 6.10	53.7 $\pm$ 9.3	1.4
<u><i>lps calligraphus</i></u>				
W	9	50.8 $\pm$ 4.16	117.4 $\pm$ 20.8	2.3
BSF	13	52.7 $\pm$ 4.19	151.1 $\pm$ 10.6**	2.9
BS	12	47.8 $\pm$ 5.65	139.6 $\pm$ 10.5**	2.9
<u><i>lps grandicollis</i></u>				
W	15	37.3 $\pm$ 5.42	108.2 $\pm$ 19.2	2.9
BSF	16	44.6 $\pm$ 6.21	148.5 $\pm$ 16.1**	3.4
BS	14	40.1 $\pm$ 5.77	147.3 $\pm$ 13.9**	3.7

* Significantly different ($p=.05$) from others within the same species. Duncan's New Multiple Range Test.

** Significantly different ($p=.01$) from others within the same species. Duncan's New Multiple Range Test.

dependent upon the beetles for dissemination and introduction into a suitable host, but also upon the activities of the beetles in the inner bark to open avenues of spread once the fungus is in the tree. When introduced into host material over a small surface area as in bolts unsuccessfully attacked in the transmission study (Table 3) and pathogenicity trials simulating bark beetle attacks (Mathre, 1964b and Nelson, 1934), the fungus may become established but does

not spread from the site of infection and sporulation does not occur. Thus the action of adult lps beetles in constructing galleries and the feeding activities of the larvae are a requisite for optimum development and spread of the fungus in the tree.

As previously mentioned, Leach et al. (1934) noted that until a brood of beetles was reared in a fungus-free log, it could not be safely concluded that blue stain fungi were not necessary for normal development of the beetles. In this study it was clearly demonstrated for the first time that all three lps species were capable of development through successive generations in pine bolts free of C. lps. lps avulsus was reared through three successive generations without observable effects when compared to beetles reared in the presence of the fungus. Similar results were obtained with lps calligraphus and lps grandicollis over four generations.

When brood development was examined more closely, it was found that lps avulsus broods free of C. lps (BSF) were comparable in size to broods reared in the presence of the fungus (BS). Additionally, no differences were detected between length of egg gallery, brood mortality, brood composition (rate of development), or pupal weight of the two brood types. The number of eggs laid by BSF and BS females after 30 days was not significantly different. Thus, it is apparent that C. lps has no effect on brood development and fecundity of lps avulsus under laboratory conditions.

It was also apparent that C. lps had little or no effect on brood development and fecundity of lps calligraphus and lps grandicollis although the data were not as conclusive as with lps avulsus.

Ips calligraphus females free of C. ips (BSF) constructed longer egg galleries and produced significantly larger broods than females (BS) in the presence of the fungus. Furthermore, BSF females laid significantly more eggs than BS females after 30 days. Conversely, however, BS Ips calligraphus broods appeared somewhat more advanced than BSF broods at 5 and 15 days. This apparent increase in rate of development was not consistent throughout the observational period, and at 10 and 20 days the relative composition of the broods was comparable. No explanation (other than random error) can be given for the significant differences between brood size and fecundity of BSF and BS beetles. It should be pointed out that in both instances the differences barely met the minimum required for significance and were possibly due to chance. Owing to the small number of broods examined and the inconsistency of the data, it can neither be assumed that the absence of C. ips results in construction of longer egg galleries and increased fecundity, nor the presence of the fungus resulted in more rapid brood development. Considering all measurements made on the various aspects of brood development and fecundity, it was evident that the fungus has little or no effect on the beetle.

With the exception of brood composition, no differences were found between BSF and BS Ips grandicollis broods. At 5 and 20 days, the composition of the broods was comparable, but at 10 days BSF broods appeared somewhat more advanced than the BS brood. The situation, however, was reversed at 15 days, and BS broods contained more individuals in the later stages of development. With this aspect

of brood development and fecundity being the only area in which differences were detected and again due to the inconsistency of the differences, there is little reason to suspect that C. ips is a factor in brood development and fecundity of ips grandicollis.

Although the results of this investigation clearly indicated that C. ips has little or no effect on brood development and fecundity of the three ips species studied, the question still arises as to the applicability of these findings to field conditions. It is well documented in the literature that Ceratocystis infection resulted in reduction of the water content of the xylem and phloem. Shepard and Watson (1959) further demonstrated the fungi indirectly reduced resin production by causing collapse of the resin-secreting epithelial cells. As previously mentioned, many authors believed reduced water content and resin flow were necessary to create conditions favorable for brood development by the beetles. The point in question, then, is whether C. ips is capable of lowering the moisture content and o.e.p. of a tree, which at the time of attack was too high, to a level suitable for brood development. Although no field studies were conducted, observations in the laboratory indicated C. ips did not become established rapidly enough to significantly influence moisture content or resin flow in the early stages of brood development. When forced to attack bolts with a high phloem moisture content, beetles of all three species, in the presence or absence of the fungus, were equally "pitched out." In some bolts, the adults became established, but the young larvae were "drowned" in resin soon after eclosion. Under field conditions, therefore, it appears unlikely that C. ips

would be a significant factor in the establishment of Ips broods. The possibility that C. ips or other Ceratocystis species would prevent, as a result of excessive environmental moisture, a sudden increase in moisture content and o.e.p. in trees with well-established broods is not excluded, however.

In addition to comparing brood development of the three Ips species in the presence or absence of C. ips, the maintenance of wild (W) colonies of beetles afforded a comparison of BS and BSF broods with broods enjoying their "normal" complement of associated organisms. As previously noted, W broods of both Ips grandicollis and Ips calligraphus did not fair as well as the BS and BSF ones. These differences were attributed to parasites; namely, parasitic mites and nematodes and pathogenic fungi. Wild Ips avulsus broods, on the other hand, appeared more vigorous than BS and BSF broods. The broods were larger and development was more rapid. Wild Ips avulsus pupae weighed significantly more than BS and BSF pupae, and the fecundity of W females was greater. Since no basic differences between BS and BSF Ips avulsus broods were exhibited, the apparent increased vigor of the W broods did not appear to be correlated with the presence or absence of C. ips.

The consistency of the difference between W and BS and BSF Ips avulsus beetles with respect to brood development, pupal weight, and fecundity suggested that some factor or factors were missing from the BS and BSF bolts. Although determinations were not made, two organisms were consistently isolated from the gallery systems and adjacent tissues in W Ips avulsus bolts; a yeast and a fungus,

probably Tuberculariella ips Leach, Orr, and Christensen. Callahan and Shifrline (1960) suggested that yeasts played a role in the nutrition of bark beetles. They noted that all species of bark beetles have associated yeasts and that the yeasts were eaten by both larvae and adults. Callahan and Shifrline added further that it has not been demonstrated that "yeast-feeding" is not a prerequisite for fertility of the adults. The results of this study clearly refute Callahan and Shifrline's hypothesis. Brood development, fecundity, and fertility of both ips calligraphus and ips grandicollis were not adversely affected when the beetles were reared in the absence of yeasts. Based upon observations made during this study, however, the above statement cannot be made for ips avulsus since broods in the presence of yeast as well as other organisms, exhibited more rapid development and larger numbers. The yeast associated with ips avulsus appeared to be the same as that associated with the other ips species; thus it is most unlikely that yeasts were responsible for the increased vigor of ips avulsus broods.

In addition to Ceratocystis ips and the undetermined yeasts, another fungus was consistently found associated with ips avulsus. The growth habits and structural characteristics of the fungi were similar to those given by Leach et al. (1934) for Tuberculariella ips. The fungus was most frequently observed in the pupal chambers where fructification took place. By the time callow adults were present the walls of the pupal chamber were lined with a white mass of conidia (Figure 12). The fungus no doubt served as a food source for ips avulsus adults as newly emerged beetles were observed

Figure 12. Ips avulsus pupal chamber covered with masses of conidia, probably Tuberculariella ips. Approx. 5X.

"grazing" on the fungal matt. Most individuals did not stray from the pupal chamber until emergence from the brood tree and apparently restricted their pre-emergence feeding to the fungus.

Francke-Grosmann (1963) stated that among phloem-inhabiting Scolytidae, some cases of association between fungi and beetles are known which can be called ambrosia because they indicate existence of true symbiosis. As one example, she cited the association between Trichosporium tengens var. macrosporum Francke-Grosmann and Ips acuminatus Gyll. In this case the fungus possessed nutrient-rich, globose conidia which completely cover the gallery walls. The young beetles fed on the fungal mass which, according to Francke-Grosmann, no doubt served as important nutrients for the young beetles. Leach et al. (1934) noted that Tuberculariella ips resembled the so-called "ambrosia" fungi by possessing large globose conidia rich in nutrients. The relationship of T. ips to Ips plini and Ips grandicollis in the United States (Leach et al., 1934) and Ips sexdentatus in Europe (Siemaszko, 1939) then appears very similar to that described above. The fungus in question in this study, probably T. ips, was only found in association with Ips avulsus. Whether its presence was responsible for the increased vigor of Ips avulsus brood must remain problematical. The dependence of Ips avulsus upon the fungus for nutritive factors, if any, was not obligatory since beetles were successfully reared in its absence. Additional data are necessary before the ecological impact of the fungus on the development of Ips avulsus can be assessed.

CONCLUSIONS

Results of this study allow the following conclusions to be drawn:

Ceratocystis lps was the only member of the genus consistently associated with lps bark beetles occurring in Florida. Another fungus, probably Tuberculariella lps, was frequently associated with lps avulsus, but not with lps calligraphus and lps grandicollis.

Ceratocystis lps was transmitted by both males and females of the three lps species. Adults were both externally and internally contaminated with the fungus. Larvae, particularly the later instars, were also internally contaminated. lps avulsus larvae demonstrated a higher incidence of contamination than lps calligraphus and lps grandicollis.

Ceratocystis lps had no influence on the mass attraction phenomenon of lps calligraphus males.

Pine bolts inoculated with Ceratocystis lps prior to attack by the three lps species proved unsatisfactory as breeding material.

The presence, or absence, of Ceratocystis lps had little or no effect on brood development and fecundity of lps avulsus, lps calligraphus and lps grandicollis. Larvae were reared on semi-artificial medium without adverse effects when compared to broods reared in bolts in the presence of Ceratocystis lps. The three lps species were reared through successive generations in bolts free of

Ceratocystis ips without observable effects on vigor and fecundity when compared to beetles in the presence of the fungus. ips calligraphus and ips grandicollis in the absence of Ceratocystis ips or in the presence of Ceratocystis ips alone produced larger brood with lower mortality than wild beetles with the normal complement of micro-organisms. These differences were attributed to parasites present in wild populations only.

Wild ips avulsus broods were larger and development was more rapid than broods in the absence or presence of Ceratocystis ips. The presence of a fungus, probably Tuberculariella ips, with the wild broods was suggested as a possible factor contributing to these differences since the fungus resembled the "ambrosia" fungi. ips avulsus adults were observed feeding on the fungus, but their dependence upon it for nutritive factors was not obligatory as beetles were successfully reared in its absence.

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BIOGRAPHICAL SKETCH

William C. Yearlan, Jr., was born May 20, 1937, at Lake Village, Arkansas. He attended elementary and secondary schools at Lake Village, Arkansas, and was graduated from Lakeside High School in 1955.

He attended Arkansas A & M College, College Heights, Arkansas, from September, 1955, until June, 1957. He transferred to the University of Arkansas in January, 1958, where he received the degree of Bachelor of Science in Agriculture in January, 1960. Upon receipt of the Crossett Research Fellowship, he entered the Graduate School, University of Arkansas in January, 1960, and was awarded the Master of Science Degree with a major in Entomology in January, 1961.

On June 30, 1960, he married the former Marguerite LaVerne Robertson of Eudora, Arkansas. They have no children.

He entered the University of Florida in February, 1961, and was granted a research assistantship with the Department of Entomology. In April, 1962, he accepted a research assistantship with the Department of Entomology, Florida Agricultural Experiment Station. In the summer of 1964, he was granted a research fellowship from the Southern Forest Disease and Insect Research Council.

In May, 1965, he was appointed to the staff of the Department of Entomology, University of Arkansas, as an Assistant Professor.

At present he is a candidate for the Degree of Doctor of Philosophy.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Date June 21, 1966

Dean, College of Agriculture

Dean, Graduate School

Supervisory Committee:

Co-Chairmen

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