TRICHOSPORIUM SYMBIOTICUM, N. SP., A WOOD-STAINING FUNGUS ASSOCIATED WITH SCOLYTUS VENTRALIS[1]

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INTRODUCTION

During the past several years an engraver beetle (*Scolytus ventralis* Lec.) has caused the death of many white firs (*Abies concolor* Lindl. and Gord.) throughout the Sierra Nevadas of California. The galleries are confined to the cambial region, and the eventual death of the infested tree is the result of girdling.

A brown discoloration has been known to be commonly associated with *Scolytus ventralis* tunnels, but it was not until 1930 that the cause of the stain was discovered. In that year an investigation was started by the Division of Forest Insects, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, on the insects infesting white firs, and Struble [3] isolated a fungus from a number of the stained areas. Inoculations with the isolated organism produced a similar brown discoloration in the wood and inner bark of uninfested trees.

Subsequent to this work and at the invitation of the Division of Forest Insects, the writer carried on additional studies of the fungus in an attempt to determine its role in the development of beetle broods. The results of these studies and a description of the fungus are given in the present paper. Special effort has been made to determine the pathogenicity of the fungus to white fir and the manner in which it is transmitted from tree to tree.

REVIEW OF LITERATURE

In 1931 Rumbold (*13*) [4] reviewed the literature relating to bark beetles and blue-staining fungi. In her own investigations she established the fact that blue stains are frequently associated with species of *Ips* and *Dendroctonus*. Grosmann (*7*) studied Ipids and concluded that the different associated fungi have the same requirements for living as the beetles but that the insects are important carriers of blue stain and related organisms. Investigators have shown that bark beetles frequently act as direct carriers of infection.

Ceratostomella (*Graphium*) *ulmi* (Schwarz) Buisman, which causes the serious Dutch elm disease, seems to be the only wood-staining fungus so far reported to be associated with *Scolytus* beetles. Wollenweber

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[3] STRUBLE, G. R. THE FIR ENGRAVER BEETLE AND ASSOCIATED INSECTS IN WHITE FIR. PRELIMINARY REPORT. U. S. Dept. Agr., Bur. Ent. 1931. (Unpublished.)

[4] Reference is made by number (italic) to Literature Cited, p. 538.

and Stapp (*14*) suggested that the frequent occurrence of *G. ulmi* in connection with *Scolytus* galleries may indicate that these beetles aid in spreading the disease, although actual proof was not obtained. Betrem and collaborators (*3*) showed, however, by isolations that *S. scolytus* Fab. adults actually carry the spores of *G. ulmi* both internally and externally. Since the spread of the Dutch elm disease to the United States, Beattie (*1*) has reported that May and Fowler isolated *G. ulmi* from imported elm logs containing *S. scolytus* broods. This interception was later supplemented by others and strongly suggests that these beetles may have played an important part in introducing the disease from abroad.

In some instances a symbiotic relationship between fungi and beetles has been indicated. Hubbard (*9*) in his study of ambrosia beetles showed that an interdependence does exist. For bark beetles true symbiosis is unusual.

MATERIALS AND METHODS

In order to secure an abundant supply of recently infested white fir material and newly emerged beetles, field laboratories were established in close proximity to the forest entomology camps where studies of *Scolytus ventralis* were at the time being pursued. The camps were situated at different localities, at elevations of 3,000 to 5,000 feet. Occasional material collected from other areas was also utilized in the course of the study.

The investigation so far has been confined to white fir, although *Scolytus ventralis* also infests other species, such as red fir (*Abies magnifica* Murr.), in this same section. Since the latter species occurs at higher elevations, the beetle emergence period is considerably shorter and infestations are less favorable for continued study.

Material for a close study of the fungus that causes the stain was obtained from freshly infested firs. Individually stained areas of xylem and inner bark were prepared for culturing by cutting away surface contamination with a flamed scalpel, thereby exposing fresh stain. Slivers were removed aseptically and planted on malt agar (2.5 percent malt extract to 2.5 percent agar) in Petri dishes. Isolations were later transferred to test-tube slants.

THE STAINING FUNGUS

ISOLATION

Stained material was cultured from 100 different galleries during the course of the study. A summary of the isolation results is presented in table 1.

TABLE 1.—*Isolations from Scolytus ventralis galleries in Abies concolor*

Material	Location of tree	Total galleries cultured	Cultures from galleries showing—		
			Causal organism	Other fungi	No organism
		Number	*Number*	*Number*	*Number*
Recent stain in inner bark	Stanislaus National Forest	16	14		2
Recent stain in xylem	do	10	8	2	
1-year-old stain in xylem	do	11	6	3	2
Recent stain in inner bark	Sequoia National Forest	8	8		
Recent stain in xylem	do	32	26	4	2
Do	Sierra National Forest	13	10	2	1
Recent beetle entrance holes	do	10	5	5	
Total		100	77	16	7

From a total of 100 isolations attempted, 77 produced cultures of the causal organism, 16 contained other fungi, and 7 were negative. The fungi other than the causal organism did not occur consistently. A few of the more unusual types were reduced to pure cultures for use in future tests to determine their staining possibilities.

In a few cases conidiophores of the fungus were observed as delicate, whitish hyphae in vacated *Scolytus ventralis* galleries. Because of their fragile character it is difficult to detect such formations regularly, and cultures were needed for verification.

Unlike the common blue stains in trees, the brown discoloration does not penetrate deeply into the sapwood and is confined to the annual ring in which the galleries are made.

A comparison of the fungus with Struble's isolations showed that the strains were identical in cultural as well as in microscopic features.

IDENTITY OF THE FUNGUS

There is general agreement that the fungus belongs in the Dematiaceae and to the genus *Trichosporium* Fr. No description has been found in the literature which can be applied to the organism referred to in this paper. It therefore appears necessary to designate the fungus as a new species.

TECHNICAL DESCRIPTION

Trichosporium symbioticum, n. sp.

Hyphis septatis, pallidis vel fuscis brunneis, intercellularibus, mediocribus 3.0μ diam.; conidiophoris ramosis, septatis, hyalinis, 1μ–1.5μ crassitudine; conidiis hyalinis, subglobosis, sessilibus, apice insertis, capitulatis et subterminalibus pleuracrogenis, 1.8μ–2.4μ diam.

Hyphae septate, colorless to brown, intercellular, averaging about 3μ in diameter. Conidiophores septate, colorless, 1μ–1.5μ in diameter, and bear clusters of spores inserted on the apices or occasionally subterminally of the main axis or on secondary or tertiary branches. Spores attached at the tips, hyaline, subglobose, 1.8μ–2.4μ in diameter. Associated with *Scolytus ventralis* Lec. galleries, causing a brown stain in the cambium and adjacent xylem and phloem of *Abies concolor* Londl. and Gord. in California.

In malt-agar cultures, submerged mycelium eventually fuscous brown, averaging 6μ in diameter, conidiophores colorless, clustered, erect, septate, 1.5μ–2μ thick, spores variable in size, 1.5μ–4μ in diameter.

On Abietineae (*Abies concolor* Lindl. and Gord.), California.

Type locality.—Strawberry, Stanislaus National Forest. Stan. W–201 wood and bark, herbarium, Division of Forest Pathology, San Francisco, Calif. Parts of type also deposited in mycological collections, Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture. Test-tube and plate cultures deposited with each collection.

The two distinguishing features that segregate this species from most other described species of *Trichosporium* are the colorless spores and their minute size. Descriptions of species that have colorless spores were found inapplicable, mainly because of their considerably larger spore size or in some cases because other characters as well did not agree, such as the color of the hyphae and the shape of the spores. A few of the descriptions given in the literature were not adequate for accurate comparison.

CULTURAL CHARACTERISTICS

Pure cultures of the fungus were grown on malt, potato-dextrose, corn-meal, starch, and modified Czapek's [5] agar. On malt agar

[5] KH_2PO_4 was used in place of K_2HPO_4 since an acid medium is more satisfactory for growing wood-inhabiting fungi.

which was the most favorable medium used, two kinds of mycelium were produced, (1) a white flocculent aerial mycelium, bearing conidiophores and spores, and (2) dark submerged hyphae, varying from cinnamon drab to fuscous brown in color. The submerged hyphae do not show noticeable coloration until the culture is 10 to 15 days old. The older the culture the darker the submerged mycelium becomes. On other agars the mycelium shows different degrees of coloration, all less distinct than that on malt agar, and no coloration on the Czapek's agar. Figure 1 shows the characteristic development of the mycelium under cultural conditions.

FIGURE 1.—*Trichosporium symbioticum* on malt agar, showing white flocculent air mycelium and dark submerged hyphae. Natural size.

MORPHOLOGY

In malt agar (pH 5.5) the dark-brown submerged mycelium is plainly septate and in old cultures the hyphae may obtain a maximum width of 14μ, with an average of 6μ. The whitish aerial hyphae are more delicate, ranging from 1.5μ to 2μ in width, and are also septate. Conidia are distinct from the conidiophores and occur in capitate clusters at their tips but occasionally subterminally as well. The spores are attached by the tips and are permanently hyaline, subglobose to ovoid in shape, and 1.5μ to 4μ in diameter. The conidiophores often divide into two or more branches, each of which may bear a capitate group of spores on the apices as illustrated in figure 2.

Microtome sections of infected wood, stained by the Cartwright (*6*) and Hubert (*10*) methods, when examined microscopically showed the presence of *Trichosporium symbioticum* hyphae within the tracheids,

and occasionally conidiophore and spore formations were also detected. Figure 3 is a photomicrograph showing *T. symbioticum* hyphae and spores within the tracheids of *Abies concolor* wood. The hyphae effect an entrance into the tracheids through the bordered pits and not directly through the cell walls. Hyphae also enter the medullary ray cells through the pits but are not as typically congregated here as is frequently the case with other wood-staining organisms such as *Ceratostomella* spp. Hyphae have been seen in the phloem parenchyma, but only indistinctly.

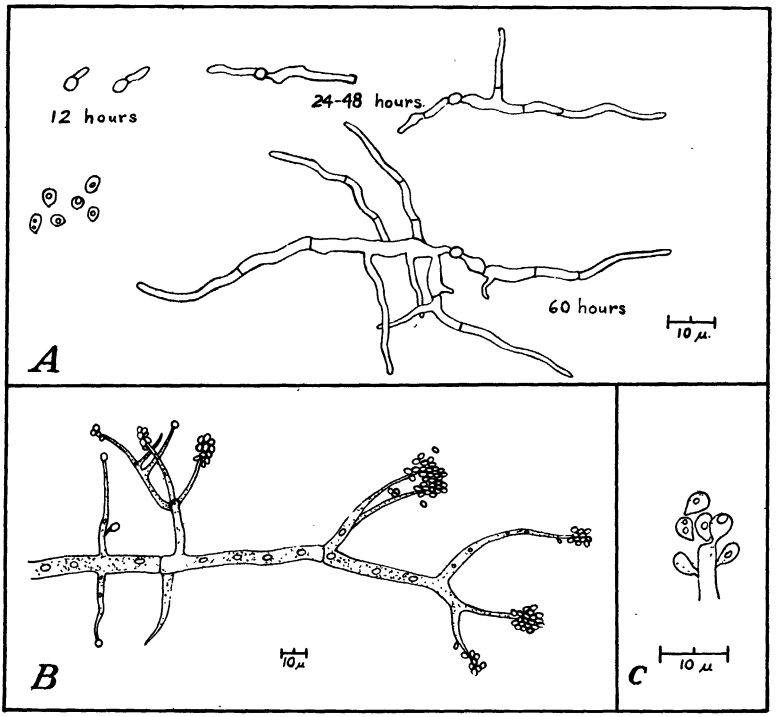

FIGURE 2.—*Trichosporium symbioticum* on malt agar: *A*, Spore germination and mycelial growth at 22° C.; *B*, typical aerial mycelium and conidiophores; *C*, enlarged tip of a conidiophore, showing manner of spore attachment.

In the tracheids of *Abies concolor* wood the maximum width of mycelium observed is approximately 8μ, averaging about 3μ. The spores produced in the tracheids are subglobose and vary from 1.8μ to 2.4μ in diameter. The sizes of the spores and hyphae do not differ as much within the tracheids as when the organism is grown in culture media.

OCCURRENCE OF STAIN IN GALLERIES OF SCOLYTUS VENTRALIS

To determine the frequency of stain occurrence in relation to *Scolytus ventralis* galleries, five infested trees were examined after they had been felled and the bark had been removed. These trees ranged from 8 to 30 inches in diameter. In all, 532 galleries were inspected macroscopically in consecutive order, and the brown discoloration was plainly evident in every case.

Special attention was given to evidences of overcome and unsuccessful beetle attacks, generally characterized by isolated galleries, to determine whether the brown discoloration was noticeable, thereby indicating the presence of *Trichosporium symbioticum*. In no instance was the coloration found to be entirely lacking, although the areas stained in unsuccessful attacks appeared to be less extensive than in successful infestations.

In trees where the beetle attacks are but few or have been unsuccessful, the stain is soon overgrown by newly formed xylem so that it becomes buried under successive layers of annual growth. Years later, when such trees are felled and bucked, the stained areas may become evident on the cross cuts. The date of the attacks may be readily established by counting the number of rings that have been formed over the stain (fig. 4).

FIGURE 3.—Hyphae and spores of *Trichosporium symbioticum* within tracheids of *Abies concolor* wood. × 450

The trap trees [6] used by the entomologists to attract *Scolytus ventralis* provided another means of studying the frequency of the occurrence of the fungus with individual egg galleries. Several weeks after the attacks were made the bark was removed from seven different trap trees and 999 galleries were examined successively. Stain was plainly evident in all but 28, and these had been formed very recently. The number of trap-tree galleries inspected plus the number of galleries found in the infested firs makes a total of over 1,500 attacks examined. The stain was unquestionably present in all but 2 percent of the galleries, and the recent origin of these may explain its visual absence, since cultural isolations have already shown that the fungus was probably present in these doubtful cases.

At the time of the examination of the trap-tree attacks, measurements were made of the extent of the stain. Estimates of the approximate age of the egg galleries were based on their length. It was found that the discoloration spread longitudinally at an average rate of approximately 3 mm a day. It was possible to follow the rate at

[6] In this investigation a trap tree consisted of a purposely felled, living white fir that was left unlopped on the ground. Unlopped trees are especially attractive to *Scolytus ventralis*, and by using this method forest entomologists secured abundant infestations at will.

which the stain spread for about 1 month, but after that time the discoloration of adjacent galleries ran together (fig. 5). The measurements indicated that individual trap trees show considerable variation in the rate at which the stain spread from the galleries.

The beetles extend their mines as the eggs are laid, and the stain is therefore first noticeable in the older portions of the galleries. Before any of the eggs hatch into larvae, however, the brown discoloration spreads longitudinally from 30 to 50 mm on both sides of the egg gallery. With this start, the stain keeps well in advance of the fastest working larvae. The writer has never found larval mines that have extended into unstained areas.

FIGURE 4.—Section of *Abies concolor* tree heavily mined with *Scolytus ventralis* galleries. Dates indicate year of attack. Notice callus formations surrounding the overcome and unsuccessful attacks. Extent of stain associated with the 1931 attacks was outlined by the writer. Photographed by George R. Struble; approximately one-fifth actual size.

It appears from these examinations that when *Scolytus ventralis* attacks white fir a brown stain is regularly associated with the galleries of this beetle. Discoloration of the same sort has never been found except in connection with beetle infestations.

THE BEETLES AS CARRIERS OF THE STAINING FUNGUS

ISOLATIONS FROM LIVING BEETLES

Infested logs placed in the Bureau of Entomology and Plant Quarantine rearing cages provided a ready supply of beetles. The adults emerged into small glass jars on the front of the cages, and 100 beetles were collected in this manner. Each insect was placed in a 50-mm Petri dish containing lukewarm malt agar, where they swam about until the agar solidified. The beetles were then removed with sterile forceps and the plates were incubated. Microscopic examinations of the plates were made periodically.

A number of different fungi were found growing in the plates, but 90 percent produced cultures of *Trichosporium symbioticum*. The most common contaminants were *Penicillium*, *Aspergillus*, and *Mucor* spp., none of which occurred consistently. In a few instances the plates contained practically pure cultures of *T. symbioticum*.

The beetles in the above investigation were not collected singly, and there was a possibility of mutual contamination. To obviate this objection 25 additional beetles were removed from the bark of infested

white fir before they emerged, and were placed in separate sterile test tubes preparatory to cultural proceedings. Later they were removed from the test tubes into lukewarm agar and allowed to swim about as previously described.

These beetles all proved to be carriers of *Trichosporium symbioticum*. It may be assumed, therefore, that *Scolytus ventralis* commonly carries the staining fungus and in this way introduces it into the egg galleries. It appears that an occasional beetle that may emerge free of the fungus is likely to become a carrier afterward through contact with other beetles.

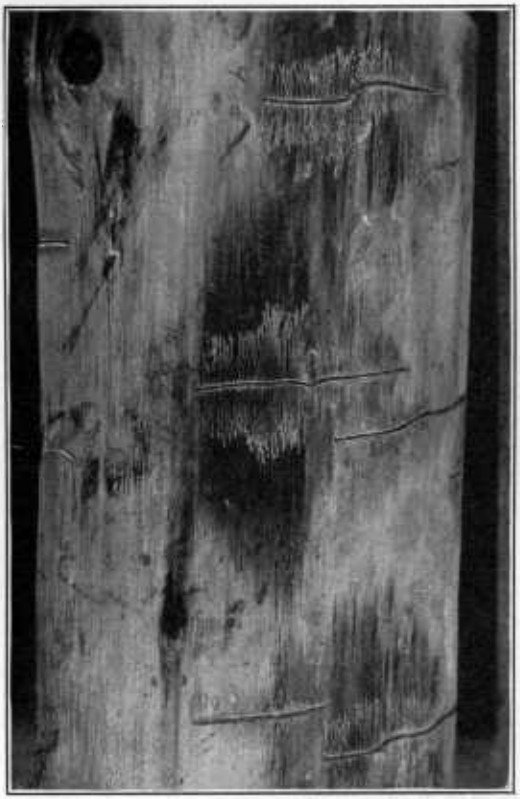

FIGURE 5.—Stain in the wood of an *Abies concolor* tree attacked by *Scolytus ventralis*. The stain is shown well in advance of the longest larval galleries, and in several cases the discoloration has spread from one colony to another, forming extensive areas. Photographed by J. M. Miller. Approximately one-fourth actual size.

ISOLATIONS FROM ALIMENTARY TRACTS

To determine whether the beetles carried *Trichosporium symbioticum* internally or externally, 50 *Scolytus ventralis* alimentary tracts were cultured by means of the following technic: Before the beetles were dissected they were sterilized according to a method used by Beckwith and Rose (*2*), who studied the intestinal flora of termites. The living beetles were placed in a U. S. P. tincture of iodine solution for 20 to 30 seconds. They were then washed in two changes of physiological saline solution and finally in two changes of sterile water. The beetles were dissected under sterile conditions by Struble. Each alimentary tract was plated immediately on malt agar by the writer. The plates were examined regularly during incubation.

Trichosporium symbioticum developed from only 4 of the 50 alimentary tracts. The small number of positive isolations indicates that the fungus is not commonly carried internally.

PATHOGENICITY TESTS

As a test of the pathogenicity of the isolated fungus, Struble [7] drilled a number of white fir trunks with a $\frac{3}{32}$-inch bit to simulate the beetle galleries, and inoculated through these openings. The writer inoculated seven trees in a similar manner, mainly to determine the

[7] STRUBLE, G. R. See footnote 3.

rate at which the stain spread. The surface of the bark was sterilized with 70-percent alcohol and the drillings were made horizontally in an ascending spiral to avoid the effect of mechanical girdling. The inoculum, consisting of *Trichosporium symbioticum* in bits of malt agar, was inserted into the drill holes with a sterile needle. Check insertions of sterile agar were alternated with the inoculations in the same trees. Both the inoculations and checks were left undisturbed for 3 to 8 weeks. Then the bark was cut away to expose the stain and in some cases the trees were felled and the bark entirely stripped from the trunks. This revealed the presence of stain very clearly (fig. 6).

FIGURE 6.—Section of *Abies concolor* trunk, inoculated by the drill method, from which the bark has been removed. On the left the checks show the absence of stain (indicated by check marks). On the right are two inoculations showing the spread of the stain in the sapwood (after 4 weeks). Approximately one-half actual size.

Measurements from a total of 145 inoculations showed that the stain spread only approximately one-third as rapidly as the discoloration associated with the beetle galleries, the daily spread averaging 1 mm.

All the checks except 5 in 1 tree remained free of discoloration. The trunk of this particular tree was enclosed with a wire screen after inoculation, and a number of *Scolytus ventralis* adults were introduced later into this cage by the entomologists, to determine whether the beetles preferred stained or unstained areas in which to establish their egg galleries. The experiment failed to settle the question, possibly because of the presence of excessive balsam,[8] which had exuded from the drill wounds and which apparently restricted the beetles from making extensive explorations. Since both the checks and the inoculations were left open, it is possible, however, that exploring beetles did introduce the fungus into the five checks.

Reisolations were taken from four of the inoculated trees and reinoculated into other unstained white fir trees. Subsequent examinations revealed the presence of the brown discoloration in all reinoculations.

[8] Preliminary tests by members of the Division of Forest Insects, Bureau of Entomology and Plant Quarantine, have since shown that white fir balsam is actually very toxic to *S. ventralis* beetles.

Several other organisms, obtained during isolation studies, were also inoculated into white fir trunks but gave negative results.

CORK-BORER INOCULATIONS

To determine whether *Trichosporium symbioticum* could kill white fir trees without the aid of the beetle galleries, it was necessary to devise a method of inoculation in which the inoculum could be placed in the cambial region in such a manner that the stain would naturally spread to form a solid band around the tree trunk. To meet this requirement and to reduce the effect of mechanical wounding to a minimum, a cork-borer method of inoculation was employed (*15*).

FIGURE 7.—*Abies concolor* tree 10 months after inoculation with *Trichosporium symbioticum* by the cork borer method. Balsam has started to flow from the base of the girdle, which was produced by the coalescence of the stain. This is tree 7 in table 2. One-third actual size.

Twenty white firs growing on the same site were selected, 10 to be inoculated and 10 to be used as checks. Considerable care was exercised in these selections to obtain check trees that were as nearly as possible duplicates of the trees inoculated. Before the inoculations were made, the portion of the stem to be inoculated was carefully washed with 70-percent alcohol.

The method consisted of using a no. 3 cork borer to cut out a series of bark disks extending entirely around the tree trunk. From one-half to 1 inch of bark was left between borings, to obviate mechanical girdling. The borer was forced through the bark down to the xylem and carefully withdrawn containing the bark disk in the barrel. The inoculum, consisting of *Trichosporium symbioticum* in malt agar, was deposited in the hole with a sterile needle, and the disk was immediately replaced by forcing it from the barrel directly into place with a plunger.

After the completion of a ring of borings, a strip of waterproof adhesive tape was placed over the disks and around the tree trunk. This held each disk in position and prevented as far as possible the detrimental effect of drying out.

Since the fungus, as previously indicated by the stain spread, has only a limited lateral growth, it was necessary to arrange the inoculations in more than one ring. These were made about 2 to 3 inches apart, with the borings of the lower ring alternating with those of the

ring directly above. In this way the uninoculated areas between the borings would be more certainly invaded by the fungus hyphae as they extended longitudinally.

The checks were treated in the same manner as the trees inoculated, except that sterile malt agar was used instead of the inoculum.

The results secured from the cork-borer inoculations are presented in table 2, along with other essential data. The condition of the trees was judged largely on the general appearance of the crowns. Final examinations were made 16 months after the beginning of the tests. Examinations of the inoculated trees were made by peeling off the bark from the inoculated portions.

Eight months after the beginning of the tests the bark adjacent to the inoculated areas was much cracked and there was a considerable flow of balsam. On several of the trees definite cankers had formed and callus formation had started. All of the check trees at the time appeared normal, and only a very slight superficial cracking of the bark could be detected, with no external balsam flow. By midsummer of the year following the initiation of these tests, some of the inoculated trees had failed to produce new needles and a few dead branches could be seen in the crowns. The infected trees all showed pronounced callus formations accompanied by definite cankers and heavy flow of balsam from the region of inoculation.

TABLE 2.—*Results of cork-borer inoculations after 16 months*

Tree no.	Diameter breast high	Vigor	Borings	Bands	Condition [1]
	Inches		*Number*	*Number*	
1	4½	Medium	13	2	Dead.
2	3½	do	11	2	Normal.
3	5	do	13	3	Several dead branches.
4	2½	do	6	2	Dying.
5	2	Poor	5	2	Normal.
6	3½	Medium	11	1	Do.
7	3	Poor	14	2	Dead.
8	3½	Medium	11	2	Normal.
9	4	do	12	2	Do.
10	4½	do	16	2	Dead.

[1] Condition of all checks was normal.

Detailed examinations of all the inoculated trees showed that the cambium was killed as the fungus advanced and that when the stain had coalesced into a solid band extending completely around the tree trunks an effective girdle was formed. It was also found that when the inoculated trees did not show indicative dead branches in the crown the stain had failed to coalesce in one or more places on the trunks; hence the girdles were not complete, and the trees survived. It should be mentioned, however, that before any of the inoculated trees succumbed *Scolytus* beetles had attacked the trunks above the girdles and probably hastened the death of the trees. Figure 7 shows the appearance of an inoculated tree trunk at the conclusion of the test. The checks remained in normal condition throughout the entire period.

From these tests it may be concluded that *Trichosporium symbioticum* is definitely pathogenic. Mechanical wounds, such as those pro-

vided by the beetle galleries, may aid the fungus in becoming established but are not essential for the extension of the hyphae. The fungus, spreading ahead of the galleries in beetle-infested trees, kills the cambium as it advances. When the stain coalesces to form large areas, spreading from one gallery to another, it produces a more effective girdle than would the galleries alone.

Others have also shown that wood-staining organisms may be parasitic on the host. Münch (*11*) concluded that under certain conditions the bluing fungi, with which he worked, may cause the death of pine trees. Nelson and Beal (*12*), working with southern pine in this country, showed that the blue stains associated with *Dendroctonus frontalis* Hopk. may play an important part in killing infested trees. The organisms studied by the above workers did not belong in the genus *Trichosporium*, but other investigators have indicated certain of these species to be parasitic as well. Butler (*5*) found *T. vesiculosum* Butl. associated with the death of casuarina trees in India. He found that the hyphae lie within the vessels and bore their way through intervening cell walls. In other instances, species of *Trichosporium* have been indicated to be parasitic, such as *T. parasiticum* Dearn. and Bisby (*4*) on *Amelanchier alnifolia* Nutt. leaves.

Since stain development precedes the appearance of the larvae, it may be that the fungus aids the brood by adjusting localized conditions. This seems to be particularly pertinent in isolated galleries, such as overcome attacks, where the host tree remains alive but the larvae thrive.

The killing of the cambial region probably restricts the flow of balsam, or the hyphae of *Trichosporium symbioticum* may render certain food products more digestible, as shown by Heitz (*8*) and others for other fungi. The effect of the stain on moisture conditions may also be of particular importance, as suggested by Struble,[9] since the larvae have never been found in unstained material. It appears desirable, therefore, to determine what effect the fungus may have on moisture conditions within infected areas.

MOISTURE RELATIONSHIP TESTS

The cork-borer method was employed again to construct a partial band of *Trichosporium symbioticum* inoculations extending around only one-quarter of the circumference of several trees. Two rings were spaced 2 inches apart and the borings of the lower ring alternated with those above, as described before. Ten white firs were inoculated in this manner, and the disks were sealed in place with adhesive tape.

These inoculations were made in late June and were not disturbed for 2 weeks since a similar length of time elapses between the initiation of a *Scolytus ventralis* attack and the appearance of the first larvae. At that time the adhesive tape was removed and a 1-inch arch punch [10] was driven through the bark between the inoculation rings and into the sapwood where the stain would be coalesced. In this manner a core of stained wood and overlying bark was readily removed from five inoculated trees and to prevent desiccation was

[9] See footnote 3.

[10] An arch punch is a steel tool used in the leather trade. It has a sharp, hollow, circular blade over which the handle arches; hence the name.

wrapped in tin foil before being deposited in a container which was then tightly closed. Similar cores of fresh unstained wood were extracted about an inch from the end of the inoculated areas. Since the discoloration does not penetrate deeper than the current growth ring, the specimens were trimmed down to the thickness of one ring and the bark was removed before weighing. The cores were dried at 100° C. until a constant weight was obtained for each sample, and the percentage of moisture was determined on a dry-weight basis. Table 3 summarizes the comparative results of the final weighings.

TABLE 3.—*Comparison of moisture in stained and unstained white fir wood 2 weeks after inoculation*

Tree no.	Diameter breast high	Unstained wood			Stained wood			Difference in moisture
		Wet weight	Dry weight	Moisture [1]	Wet weight	Dry weight	Moisture [1]	
	Inches	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*	*Percent*
11	9	1.321	0.434	204	1.128	0.569	98	−106
12	7	1.388	.484	187	.618	.320	93	−94
13	6	1.474	.482	206	1.550	.672	131	−75
14	8	1.753	.599	193	1.788	.760	135	−58
15	7	2.134	.684	212	2.050	.920	123	−89
Average	7.4	1.614	.536	200	1.427	.648	116	−84

[1] Percentage of moisture based on dry weight.

After 2 months it was found that the moisture in stained samples from five other white firs still averaged 88 percent less than that of unstained wood taken from the same trees.

It is known that moisture fluctuates considerably in individual trees, depending upon the time of the day and the season, and that there may also be variations even in the same tree trunk, particularly with reference to the circumference. These percentages are, therefore, presented as approximate rather than absolute. The fact remains, however, that *Trichosporium symbioticum* does reduce the moisture conditions locally and that the beetle larvae develop where moisture is less than in adjacent unstained areas, since their galleries occur only in association with the discoloration. It is not impossible that the fungus may consequently render conditions more favorable for the successful development of the larvae and particularly aid in the preservation of isolated colonies.

CONCLUSIONS

On the basis of the data collected, the writer suggests that the relationship of *Trichosporium symbioticum*, n. sp., to *Scolytus ventralis* Lec. may be one of mutual advantage.

The beetle benefits, since the fungus kills the cambium as it advances and thereby assists in overcoming the host tree. The fungus reduces the moisture content of the cambial region ahead of the larvae and in this way may render conditions more favorable for their development.

The fungus benefits, since it is carried by the beetles to new and suitable substrata, presumably otherwise unattainable, where it is able to maintain and reproduce itself. The association appears to be symbiotic.

SUMMARY

A fungus has been frequently isolated from a number of brown-stained areas associated with *Scolytus ventralis* Lec. galleries. The isolated organism has been determined as *Trichosporium symbioticum*, n. sp.

The stain is regularly associated with *S. ventralis* galleries in white firs and has always been observed to precede the appearance of the larvae.

S. ventralis adults carry *T. symbioticum*, mostly epizoically, and in this way presumably spread the fungus from infected to noninfected trees.

A cork-borer method of inoculation has shown that *T. symbioticum* is definitely pathogenic and capable of killing the cambium as it advances. In this way it may aid the beetles in overcoming the host trees. The hyphae, spreading ahead of the larvae, reduce the moisture content of the areas in which the larvae extend their mines.

The regular association of *T. symbioticum* with *S. ventralis* appears to be one of mutual benefit and suggests a symbiotic relationship.

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