The Response of Sapwood of Norway Spruce to Infection by Fomes annosus

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ABSTRACT

Examination of freshly cut Norway spruce (*Picea abies*) during all seasons of the year revealed that an alkaline, light green to olive-colored *reaction zone* was present between sapwood and the brownish central core of wood decayed by *Fomes annosus*. The *reaction zone,* which contained dead, almost starch-free parenchyma, was formed in advance of the pathogen. Bacteria and nonhymenomycetous fungi were isolated occasionally from *reaction zones,* particularly in lower parts of stems where moisture content of this tissue frequently was high.

Reaction zones darkened in color shortly after trees were felled. This color change was dependent upon oxygen, and it appeared to be mediated by oxidative enzymes produced by the host. *Reaction zone* formation was attributed to a nonspecific response to injury or infection during necrosis of parenchyma.

The *reaction zone* contained more extractives than adjacent tissues, particularly phenols with ultraviolet absorption spectra typical of lignans. *Reaction zone* and incipiently decayed wood contained more potassium, calcium, and magnesium than sound sapwood or heartwood.

Expressed sap from the *reaction zone* had a fungistatic effect on *F. annosus;* this could not be wholly explained by the high pH of this extract (about pH 8.0). *Reaction zone* and incipiently decayed wood were significantly more resistant to decay than sound heartwood and sapwood. Spruce oleoresin and one of its components, abietic acid, inhibited linear growth of *F. annosus* in vitro. Results support the hypothesis that insufficient quantities of inhibitory substances in heartwood contribute to the susceptibility of spruce to extensive central stem rot, whereas limited invasion of sapwood is due to accumulation of inhibitory substances in a *reaction zone.* Phytopathology 61:301-307.

Additional key words: host resistance.

Fomes annosus (Fr.) Karst. causes an extensive central stem rot of Norway spruce (*Picea abies* [L.] Karst.). Rennerfelt & Nacht (13) noted that fungitoxic extractives were lacking in spruce heartwood, whereas the inhibitory pinosylvins are present in pine heartwood. This, together with the inability of spruce to accumulate oleoresin in inner sapwood (6), has served as an explanation for the greater susceptibility of spruce to stem rot than pine. It is curious, however, that despite the extensive longitudinal invasion of spruce heartwood, penetration into living sapwood is indeed limited. This observation suggests that a host response may be involved in retarding spread of the pathogen into living tissue. Such a phenomenon was reported in *Pinus taeda* where substances inhibitory to *F. annosus* accumulated in sapwood in advance of fungal invasion (19).

Preliminary observations of freshly cut spruce stems revealed that a light green to olive-colored zone as much as 3 cm in width surrounds the brownish central core of decayed wood. The outer portions of this zone usually became darker in color (gray-blue to black) on the cut surface shortly after trees were felled, suggesting oxidation of accumulated phenols. *Fomes annosus* was not isolated from the outer portions of this zone, occasionally isolated from its inner portions, and consistently isolated from the brown central core. These observations are in agreement with Björkman et al. (1), who referred to this zone which readily darkens as the water-stained zone in the inner part of the sapwood. This zone will be referred to as *reaction zone* during the course of this report. Direct application of pH indicators to cross-sections revealed that the *reaction zone* was more alkaline than adjacent tissues.

The objective of this investigation was to gain information on the *reaction zone* in spruce, particularly as it may relate to resistance of sapwood to infection by *F. annosus.* A preliminary report on this research was included in an earlier publication (20).

MATERIALS AND METHODS.—*Association of reaction zone with infection by F. annosus.*—Observations were conducted on 20 naturally infected trees cut during all seasons of the year in the vicinity of Vollebekk, Norway, and three trees artificially inoculated in the vicinity of Stockholm, Sweden (artificial inoculations by A. Hyppel, August 1964; trees were felled in August 1968). To determine if *reaction zone* formation is a specific response to infection by *F. annosus,* additional observations were conducted on another tree from Vollebekk infected by *Stereum sanguinolentum* (A. & S.) Fr., one tree from Hurdal infected by *Odontia bicolor* (A. & Sw.) Bres., three trees from Stor-Elvdal infected by brown rot fungi (*Coniophora* cf. *puteana* [Schum.] Karst. and two unidentified species), and several trees from Hurdal that were artificially wounded.

The moisture content of various host tissues (sound sapwood, *reaction zone,* incipiently decayed wood, and sound heartwood) in freshly felled trees was determined periodically. Sound heartwood, subsequently distinguished from sapwood with the vital stain triphenyl

tetrazolium chloride, was obtained from uninfected trees. Moisture content was calculated as the per cent of oven dry (103 C) wt.

The pH indicators, chlorophenol red and phenol red, were streaked on freshly cut cross sections to identify zones of differing pH.

A malt extract (1.25%) agar (2%) medium was used to make routine isolations. Two other media were used occasionally to test for the presence of more fastidious microorganisms: (i) a malt-yeast extract medium described by Shigo (20); and (ii) "Eugon-broth" (Baltimore Biological Laboratories). Cultures were incubated at about 24 C and usually examined after 4, 6, and 10 days.

Factors affecting the change in color of the reaction zone.—The observed darkening of the *reaction zone* on the cut surface shortly after trees were felled suggested that either oxygen and/or light were necessary to initiate the process. Furthermore, it was not known whether these reactions were or were not enzymatic. A series of experiments was conducted to differentiate among the above possibilities. Discs were sawn from a freshly cut tree. Pairs of adjacent discs were rapidly given one of the following treatments: (i) aerobic atmosphere versus anaerobic atmosphere (container with nitrogen and alkaline pyrogallol); (ii) dark versus light (combination of artificial and daylight); (iii) steamed (45 min) versus unsteamed; (iv) water dip versus sodium azide (0.3 M) dip [the latter being an inhibitor of certain oxidative enzymes (2)]. The degree of darkening was recorded on the matched surfaces of disc pairs after 24 hr. Incubation temp was approx 25 C.

Histology and cytochemistry.—To determine the spatial relationship between living host parenchyma and fungal hyphae, tests were made for the localization of NAD diaphorase employing nitro-blue tetrazolium (NBT) as previously described (19). The location of phenoloxidases in host tissues was determined by incubating sections (30 μ in thickness) in reaction mixtures containing 1×10^{-3} M guaicol or 3.5×10^{-4} M 1-naphthol in 0.1 M phosphate buffer at pH 5.6. Checks included sections steamed first for 40 min, then placed into the complete mixture, or sections incubated in buffer without substrate. Sections were incubated at 37 C for 30 min.

A comparison of substrate specificity was made between the phenoloxidase in wood decayed by *F. annosus* and the phenoloxidase subsequently found in the *reaction zone*. Steamed and unsteamed expressed sap from the above tissues were diluted to one-third concn with 0.1 M phosphate buffer pH 5.6, sterilized by filtration (Millipore filter, pore size 0.45 μ), and placed into recesses in autoclaved and cooled agar (2%) containing one of the following phenolic compounds: L-tyrosine (1×10^{-3} M), hydroquinone (1×10^{-3} M), 1-naphthol (3.5×10^{-4} M), and catechol (1×10^{-3} M). Covered petri dishes containing the above were incubated for 12 hr at 37 C before observations on the color intensity of diffusion zones were recorded.

Starch was localized in host tissues by placing sections in a 2% aq solution of potassium iodide containing 0.2% iodine (10).

Chemical analysis.—The total amount of extractives soluble in acetone or in 70% ethanol was determined in sound sapwood, *reaction zone,* incipiently decayed wood, and sound heartwood. Extraction was conducted in a Soxhlet apparatus as previously described (19). Wood for these determinations was obtained from freshly felled bolts that were heated at 45 C for 24 hr to kill the pathogen (16) and assist in killing and drying the host.

Various chromatographic techniques were used to separate extracts. Solvent systems for thin-layer (Merck, Silica Gel-F_{254}) chromatography included: toluene, ethyl acetate, formic acid (5:4:1 v/v); and 96% ethanol, ammonia (8:2 v/v). Silica gel (Woelm, activity grade 1) was used also in column chromatography to separate the ethyl acetate-soluble fraction of extracts. Sephadex columns (G-10, 25, and 50) were used to separate the water-soluble fraction of extracts. Ultraviolet absorption spectra of fractions eluted from column and thin-layer chromatograms were determined on a Zeiss M4 Q11 spectrophotometer. An ion exchange resin (Dowex 50 W) and iodine-vapor treatment of chromatographed eluates were used in an attempt to isolate basic organic compound(s) responsible for the alkalinity of the *reaction zone*.

The content of the following elements in various host tissues was obtained: nitrogen, potassium, phosphorus, calcium, magnesium, manganese, and iron. Calcium, magnesium, and manganese were determined by atomic absorption; total nitrogen by the Kjeldahl method; and potassium by flame photometry. Iron and phosphorus were determined by colorimetric procedures (23 and 18, respectively).

The pH of various tissues was determined by the aq extract method (24) and by electrometric measurement of expressed sap with a pH meter (Radiometer).

Bioassays of host tissues and their extractives.—Expressed sap was obtained by pressing fresh or freshly frozen pieces of sound sapwood, *reaction zone,* and decayed wood in a hydraulic press. Heartwood did not contain enough moisture to permit collection of a sufficient amt of sap by this process. This tissue, therefore, was extracted with water (ca. 25 C) and the water extract was concd to the volume at which it contained a similar amt of dry matter as did expressed-heartwood sap. Sap thus obtained was sterilized by filtration (Millipore filter, pore size 0.45 μ). One ml of sap was placed into a 10-ml Erlenmeyer flask. Flasks were inoculated with a 5-mm mycelial disc taken from the periphery of a 9-day-old colony of *F. annosus* growing on malt extract agar; two isolates of the pathogen were used: 519 (from *Pinus taeda*) and 67/11 (from *P. abies*). To test the effect of sap pH on mycelial growth, the sap in some flasks was evaporated under vacuum and replaced by 1 ml of filter-sterilized phosphate buffer (0.1 M, pH 5.6). Flasks containing buffer only were inoculated to determine if the buffer alone was inhibitory to fungal growth. Flasks containing sterile distilled water were inoculated also as additional experimental controls. All flasks were incubated at approx 25 C in a humidity chamber for up to 20 days before final observations were made on mycelial growth.

The effect of spruce oleoresin on *F. annosus* was studied by suspending different concn (1%, 0.5%, 0.25%, 0.1%, 0.0%, w/v) of oleoresin in malt extract (1.5%) agar (2%). The oleoresin was dissolved in absolute ethanol and added to aliquots of autoclaved agar cooled to 50 C; the final concn of ethanol in all test mixtures, including the 0.0% check, was 1%. Mycelial discs of *F. annosus* isolates 66-138/11 (from *P. abies*) and 519 were placed on the agar surface, and linear growth of mycelium was observed at 48-hr intervals. A similar test was conducted on a component of spruce oleoresin, abietic acid ("practical grade", Fluka Chemical Co.).

A decay test employing the agar block method (2% malt extract, 2% agar) was conducted on various host tissues to compare their susceptibility to decay by *F. annosus*. Isolates 519 and 66–138/11 were used as test fungi. Five replicates of each tissue-isolate combination were incubated in Kollé flasks at approx 22 C for 14 weeks before final wt losses were determined. An analysis of variance was calculated for the resulting data.

RESULTS.—A *reaction zone* (Fig. 1, 2-A, B, C) was observed in trees infected by *F. annosus* during all seasons. In addition, *reaction zones* were observed in the artificially inoculated trees (Fig. 2-B), infected roots, trees infected by other decay fungi (including brown rot fungi) (Fig. 2-C), and wounded trees. This zone was not observed in the apical portion of the decay column where *F. annosus* was isolated only from heartwood. Observations of inoculated and wounded trees further demonstrated that *reaction zones* were produced in sapwood.

The moisture content of host tissues is summarized in Table 1. The moisture content of the *reaction zone* varied by as much as 45-140% of dry wt in the same tree; the higher moisture contents commonly occurred in the lower part of the stem where the moisture content of the *reaction zone* (e.g., 140%) was sometimes higher than that of sound sapwood (e.g., 125%).

Bacteria and nonhymenomycetous fungi were isolated occasionally from the *reaction zone*, particularly in the lower part of the stem. To test if bacteria were closely associated with *reaction zone* formation, isolations were made from sound sapwood and *reaction zone* of five trees; chips were incubated in Eugonbroth. Bacteria were isolated with equal frequency from both tissues, and in some trees from neither. The fungus most frequently isolated from the *reaction zone, Nectria cucurbitula* sensu Wollenweber (17), was observed to inhibit mycelial growth of *F. annosus* in vitro.

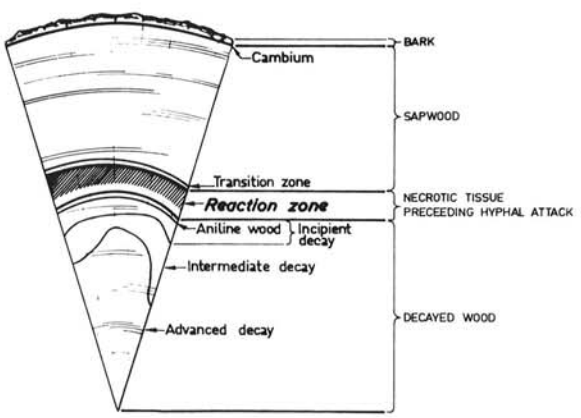

Fig. 1. Sketch of zones typically encountered in Norway spruce attacked by *Fomes annosus*.

Factors affecting the change in color of the reaction zone.—Reaction zones did not darken when they were (i) incubated in an anaerobic atmosphere (Fig. 2-A); (ii) steamed; (iii) dipped into sodium azide. *Reaction zones* in corresponding matched discs darkened when they were (i) exposed to air (in light or darkness) (Fig. 2-A); (ii) not steamed; (iii) dipped into water. These results suggest that an oxidative enzyme(s) is involved in *reaction zone* darkening. Incipiently decayed wood sometimes was bluish in color, particularly in the outer and apical portions of the decay column. This condition is known as aniline wood (1) (Fig. 1) and should not be confused with the *reaction zone* which darkens only on cut surfaces after felling.

Histology and chemistry.—NAD diaphorase activity was lacking in the *reaction zone* but present in adjacent sound sapwood, indicating that parenchyma were dead in the former and living in the latter. In several trees a marked increase in NBT diformazan deposition (and hence enzyme activity) was noted in parenchyma in sapwood immediately adjacent to the *reaction zone*. This narrow portion of the inner sapwood, which could be considered the transition zone between sapwood and *reaction zone* (Fig. 1), appeared drier and lighter in color than the remainder of the sapwood, but was similar in pH to the latter. The pathogen was not observed in the *reaction zone*, but was readily detectable in incipiently decayed wood where hyphae contained NBT diformazan.

Phenoloxidase activity was observed in parenchyma of sapwood and *reaction zone* in trees infected by *F. annosus*, a white rot fungus, as well as trees infected by brown rot fungi. Greatest activity was often observed in the ray cells of the *reaction zone* immediately adjacent to sound sapwood; however, activity was not detectable in host parenchyma in the inner portions of the necrotic tissue preceding hyphal attack (Fig. 1). Investigations with cell-free, expressed sap revealed that the phenoloxidase in the *reaction zone* was of the laccase type and that its substrate specificity was slightly different from the laccase in wood decayed by *F. annosus* (Table 2). Eighty-five cultures of bacteria isolated from trees with *reaction zones* and infected by white or brown rot fungi were assayed for extracellular phenoloxidase on malt-yeast extract agar containing 3.5×10^{-4} M 1-naphthol; results were negative in all cases. The following points serve as evidence that the phenoloxidase in *reaction zones* is a product of the host rather than the pathogen or inhabiting bacteria: (i) It is localized in host ray cells, particularly those closest to sound sapwood; (ii) substrate specificity differs from *F. annosus* laccase; (iii) *reaction zones* with phenoloxidase were observed in trees infected with fungi shown to lack phenoloxidase; and (iv) extra-

cellular phenoloxidase was not detectable from bacterial isolates.

Starch was commonly present in parenchyma of sound sapwood, but scarce in similar cells in *reaction zone,* infected wood, and heartwood.

The *reaction zone* contained considerably more extractives than other tissues, particularly those extractives soluble in the more polar solvent, 70% ethanol (Table 1). Separation of extracts by various techniques revealed that the *reaction zone* contained far more phenols than the other tissues examined. The ultraviolet absorption spectra of major components of this extract

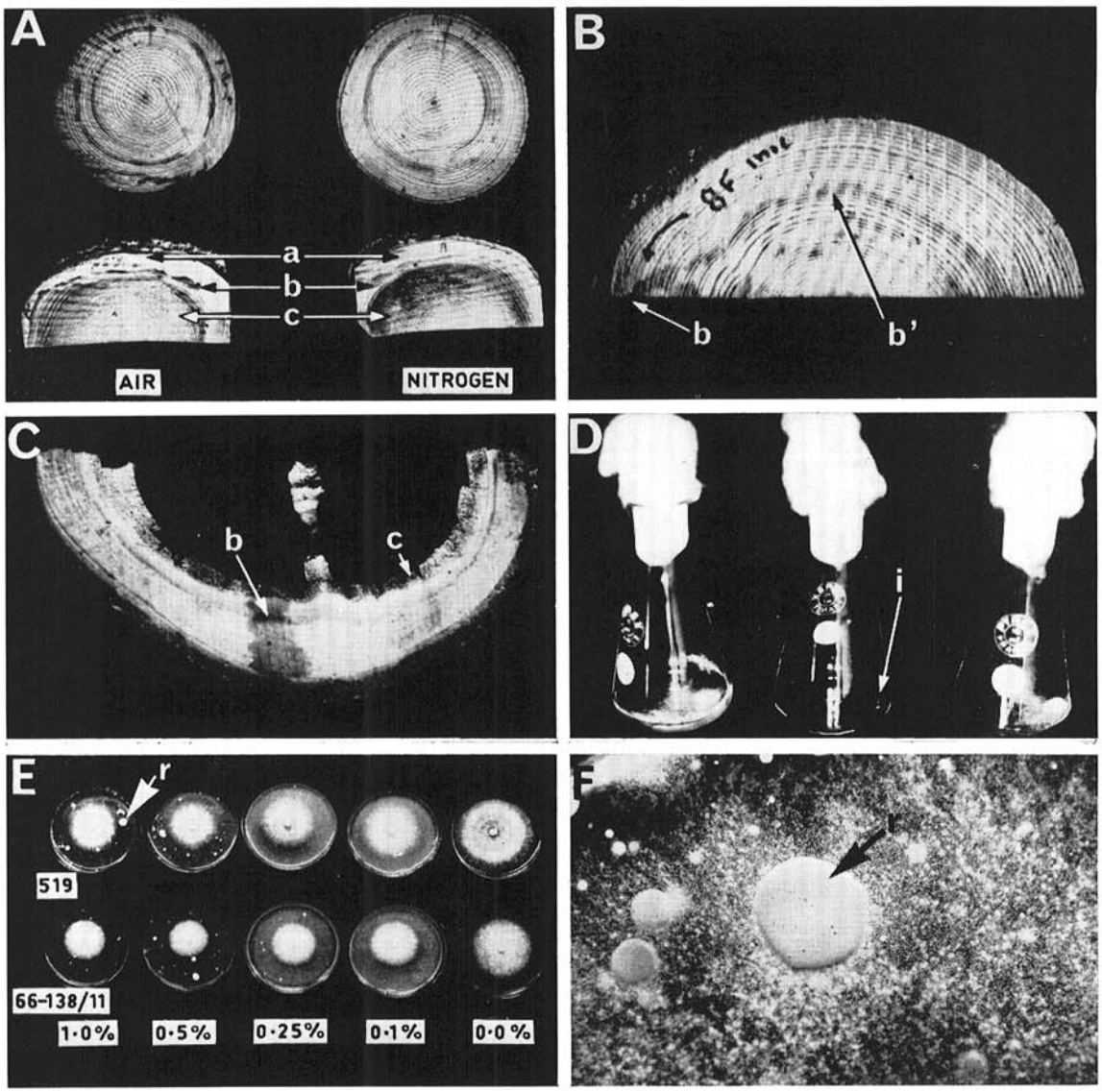

Fig. 2. A) Adjacent discs of *Fomes annosus*-infected Norway spruce 24 hr after felling and confinement in an atmosphere of nitrogen (right) and air (left); (a) sound sapwood; (b) *reaction zone*; (c) infected wood. Note darkening of *reaction zone* in discs exposed to air as compared to adjacent surfaces kept in a nitrogen atmosphere. **B)** *Reaction zone* (b,b') of Norway spruce artificially inoculated 4 years prior to felling. The inoculum dowel was in the proximity of (b). **C)** Norway spruce attacked by a brown rot fungus (*Coniophora* cf. *puteana* Schum. Karst.); (b) *reaction zone* stained with chlorophenol red. The color of this tissue after staining (red-violet) is indicative of its alkalinity compared to adjacent tissues (yellow-orange); (c) typical brown-cubical rot. **D)** Growth of *F. annosus* on expressed sap from sound sapwood (left flask), *reaction zone* (middle flask), and incipiently decayed wood (right flask) of Norway spruce. Note mycelial growth in left and right flasks (particularly former), but no growth surrounding the inoculum disc (i) in the middle flask containing sap from *reaction zone.* **E)** Growth of *F. annosus* isolates 519 and 66-138/11 on varying concentrations of spruce oleoresin suspended in malt extract agar. Note large oleoresin droplet (r) on agar containing the higher concentrations of oleoresin. **F)** Closeup of oleoresin droplet (r) shown in Fig. 2-E taken 3 weeks later. Note lack of mycelial growth on oleoresin droplet.

TABLE 1. Moisture and extractive content of sound and *Fomes annosus* infected stemwood of Norway spruce

Tissue	Moisture content		Extractives content[d]	
	Range (% dry wt)	Mean (% dry wt)	Acetone soluble (% dry wt)	70% Ethanol soluble (% dry wt)
Sound sapwood	104.0-171.2	127.4[a]	2.1	2.7
Reaction zone	38.0-157.2	79.6[b]	5.1	8.6
Incipiently decayed wood	34.8-105.2	46.4[b]	2.4	4.4
Sound heartwood	36.4- 43.7	39.8[c]	1.1	1.8

[a] Mean of 20 determinations in eight trees.
[b] Mean of 17 determinations in six trees.
[c] Mean of 6 determinations in three trees.
[d] Means of at least 2 determinations of each tissue from a typical infected tree; heartwood was obtained from a separate uninfected tree. Variation from the mean did not exceed 0.2%.

TABLE 2. Effect of steamed and unsteamed, cell-free expressed sap from *reaction zone* and wood decayed by *Fomes annosus* on various phenols as measured by the color intensity of diffusion zones in an agar medium[a]

Sap from:	Treatment	Phenol			
		L-tyrosine	Hydroquinone	1-Napthol	Catechol
Reaction zone	Steamed	—	—	—	—
	Unsteamed	—	+	++	+++
Decayed wood	Steamed	—	—	—	—
	Unsteamed	—	—	++++	+++

[a] Scale for recording intensity of colored zone: — = No reaction; + = weak; ++ = moderate; +++ = strong; ++++ = very strong.

TABLE 3. Amounts of various elements in sound and *Fomes annosus* infected stemwood of Norway spruce[a]

Tissue	N	K	P	Ca	Mg	Mn	Fe
				ppm of dry wt			
Sound sapwood	775	745	85	535	70	20	10
Reaction zone	550	3,595	35	1,085	300	100	10
Incipiently decayed wood	510	3,305	15	1,965	285	50	10
Sound heartwood	445	315	10	665	90	105	10

[a] Means of two determinations of each tissue from a typical infected tree; heartwood was obtained from a separate uninfected tree.

(peak ca. 285 mμ, trough ca. 255 mμ) was characteristic of the lignans, a group of phenols reported previously in spruce heartwood (5). A comparison of phenols present in the *reaction zone* and associated tissues will be presented in a subsequent report.

Marked increases in potassium, calcium, and magnesium were observed in *reaction zone* and decayed wood as compared to sound sapwood and heartwood (Table 3).

The pH of water extracts of the *reaction zone* was consistently between 7.0 and 7.7. Lower pH values were obtained for other tissues (pH 5.6-6.8). The following results were obtained by electrometric measurement of expressed sap from different tissues: sound sapwood, pH 5.4, *reaction zone*, pH 8.1; incipiently decayed wood, pH 5.5; and by pH indicator paper, sound heartwood, ca. pH 5.5. The ion exchange technique failed to reveal the presence of nonvolatile basic organic compounds in *reaction zone* extract. *Reaction zone* sap retained its alkalinity after oven-drying and rehydration, indicating that volatile basic organic compounds (e.g., ammonia) were not responsible for the alkalinity of this tissue. Application of dilute hydrochloric or acetic acid to cross sections, however, resulted in effervescence from the *reaction zone* only. This suggests that high pH in the *reaction zone* is due to the accumulation of inorganic carbonates.

Bioassays of host tissues and their extractives.—The fungus failed to grow on filter-sterilized, expressed sap from the *reaction zone* (Fig. 2-D), whereas growth was progressively greater on similar extracts from decayed wood, sound heartwood, and, finally, sound sapwood which supported luxuriant growth. The lack of fungal growth on *reaction zone* extract must have been due to inhibition rather than an absence of essential nutrients, because sparse mycelial growth from agar inoculum discs was observed in the distilled water checks. Inhibition, however, was fungistatic rather than fungicidal, as fungal growth occurred readily when inoculum discs were transferred from *reaction zone* sap to malt

extract agar. Since the growth of *F. annosus* apparently is limited greatly on substrates above pH 7.0 (14), high pH probably was involved in the observed fungistasis. Fungistasis occurred also when *reaction zone* sap was buffered at pH 5.6; buffer alone did not cause this effect. This indicates that high pH was not the only factor causing fungal inhibition.

Spruce oleoresin inhibited the linear growth of *F. annosus* mycelium. A concn of 0.5% suspended in malt extract agar inhibited growth by approx 50% (Fig. 2-E). The fungus did not grow over larger droplets of oleoresin on the agar surface during observations lasting several weeks (Fig. 2-F). This indicates that oleoresin can inhibit both mechanically and chemically. In a similar test, abietic acid at a concn of 0.25% inhibited mycelial growth by approx 60%.

Mean wt losses due to decay by both fungal isolates were as follows: *reaction zone,* 3.6%; incipiently decayed wood, 5.8%; sound sapwood, 8.6%; and sound heartwood, 11.8%. The *reaction zone* and incipiently decayed wood decayed significantly less (1% and 5% probability level, respectively) than did sound sapwood and heartwood. Weight losses in the last two tissues did not differ significantly.

Discussion.—The distinction as to whether the *reaction zone* was heartwood or sapwood before its conversion is critical for our understanding of the formation of this tissue. If, for example, the *reaction zone* was previously heartwood, a tissue devoid of living parenchyma, it would be logical to assume that its formation was the direct result of microbial activity. Because it appears to be formed only in sapwood, the role of bacteria in *reaction zone* formation is uncertain. Culture results indicate that formation can proceed independently of bacterial colonization. It is possible, however, that bacteria were present before isolations were made, some very fastidious species were missed, and/or that diffusates from bacteria frequently isolated from lower parts of stems are involved in formation of *reaction zones* higher up. Evans & Halvorson (4) studied a brown stain of the inner sapwood of western hemlock and concluded that it was caused by the polymerization of host-produced leucoanthocyanins by a phenol oxidase contributed by inhabiting bacteria. These authors, however, presented no evidence demonstrating that these bacteria did or could contribute the postulated phenol oxidase. In the present study, evidence was presented that strongly suggests that the phenol oxidase in the *reaction zone* was of host, and not of bacterial or fungal, origin. Since the phenols are most probably of host origin also, it seems that bacteria would have little to contribute to *reaction zone* formation, even when they are present.

There are many reports of bacteria being associated with alkaline zones in wood (9). Other studies, however, have shown that acids are formed during bacterial colonization of wood (4, 25). The suggestion that bacteria cause the alkalinity in the alkaline zones with which they are associated (9), therefore, is open to question until demonstrated. It seems more likely that the accumulation of minerals, which appears to be directly related to alkalinity (7) and phenolic extractives, is another example of the well-documented phenomenon of mobilization of metabolites in response to plant disease (26). Other factors typical of *reaction zone* formation and response to plant disease are increased activity of phenol oxidase and respiratory dehydrogenases.

The bulk of evidence, therefore, supports the hypothesis that the *reaction zone* is produced in sapwood, during necrosis of parenchyma, as a host response to injury or infection. The many differences between *reaction zone* and heartwood (content of extractives and inorganic ions, pH, color, and color change after exposure to air) suggest that differences also exist in the necrotic processes leading to their formation.

It appears that the *reaction zone* is involved in limiting the spread of *F. annosus* into sapwood. The bioassay using expressed sap not only demonstrated that inhibitory compounds were present in the *reaction zone,* but also that they probably are present in sufficient concn to inhibit in vivo. Results of the decay test, where the *reaction zone* and incipiently decayed wood were decayed less than were sound sapwood and heartwood, lend additional support to the above. Possible reasons for less decay occurring in the incipiently decayed wood than in sound heartwood are (i) the presence of residual *reaction zone* components from the past; (ii) metabolic staling factors previously introduced by the fungus; and (iii) previous depletion of readily utilizable substrates.

Luxuriant growth of the pathogen in sap expressed from sound sapwood indicates that adequate nutrients were present and that sufficient quantities of preformed inhibitors were lacking. Sound sapwood contains far more starch than the *reaction zone,* and preliminary tests indicated that the former also has a higher content of reducing sugars and amino acids than the latter. This may explain partially why sapwood of fresh stumps is colonized readily after inoculation (15). This suggests the interesting possibility that sapwood is resistant to *F. annosus* only after its conversion to a *reaction zone.*

Even though the *reaction zone* was not resin-soaked, in vitro bioassays suggest that a sufficient amount of resinous extract may have been present to contribute to its resistance. Some volatile components of oleoresin were found to inhibit *F. annosus* (3). The current paper is believed to be the first report of inhibition by a resin acid.

Microorganisms sometimes present in the *reaction zone* may also contribute to the apparent resistance of sapwood to invasion by *F. annosus.* Evidence for this was obtained from in vitro inhibition of the pathogen by *N. cucurbitula,* the fungus most frequently isolated from the *reaction zone.* Evidence was not obtained to indicate that a succession of microorganisms was necessary before invasion by *F. annosus.* This is in agreement with the notion that *F. annosus* is a pioneer invader of woody tissue (22).

Sapwood resistance to decay in living trees sometimes has been attributed to the high moisture content of this tissue (12) and consequently to an insufficient amount of oxygen for favorable growth of decay fungi.

It is unlikely that this factor played a major role in the present study, because sound sapwood was separated from the pathogen by a *reaction zone* which sometimes had a rather low moisture content (Table 1). Furthermore, previous studies indicate that growth of *F. annosus* is opt at wood moisture contents between 80-150% (11), and that it grows equally well under aerobic and microaerophilic conditions (8).

There are several distinct differences between *reaction zones* of pine (19) and spruce. The *reaction zone* of spruce differs greatly from its heartwood, whereas pine heartwood and *reaction zone* are rather similar qualitatively. Furthermore, the *reaction zone* of spruce is alkaline and that of pine acidic. In addition, the *reaction zone* of pine is resin-soaked (acetone solubles ca. 30%), whereas that of spruce is not (acetone solubles ca. 5%). Resin canals in spruce sapwood are characterized by thick-walled epithelial cells and a discontinuity of vertical and horizontal canal systems; resin canals in pine, by comparison, have thin-walled epithelial cells and an interconnected system of horizontal and vertical components (6). These differences probably contribute to resin soaking in pine *reaction zones* and its absence in spruce *reaction zones,* since previous evidence (19) indicates that resin soaking is due to the release of oleoresin from resin canals after the death of epithelial cells.

Major similarities between *reaction zones* of spruce and pine are (i) the accumulation of phenols, (ii) the constant association of this necrotic tissue with the margin between sound and infected wood; and (iii) their apparent role in protecting the sapwood from fungal invasion. The above characteristics may be used to describe and define this zone. Observations of spruce and pine strongly suggest that the pathogen seldom, if ever, invades living sapwood directly. Host parenchyma die hypersensitively or are killed by fungal metabolites in advance of fungal penetration. It is during necrobiosis of these cells that *reaction zones* are formed. As the fungus slowly penetrates this tissue, the host continues to respond by producing additional protective layers. The term *reaction zone* seems appropriate for this dynamic tissue; I suggest that this terminology be applied to similar tissues in the future.

In conclusion, it appears that the lack of sufficient quantities of inhibitory substances in spruce heartwood contributes to the susceptibility of this species to extensive central stem rot caused by *F. annosus*. The limited invasion of sapwood, despite extensive heart rot, seems to be due to the accumulation of inhibitory substances in a *reaction zone*.

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