Factors affecting infection of balsam fir (*Abies balsamea*) by *Stereum sanguinolentum* in Quebec

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L'auteur analyse les résultats d'une étude, effectuée dans le Québec pendant trois ans, sur les facteurs qui régissent l'infection du sapin baumier (*Abies balsamea* (L.) Mill.) par le *Stereum sanguinolentum* (Alb. et Schw.) ex Fr. La dissémination et la germination des spores se produisent pendant des périodes humides étendues et lorsque la température se maintient entre 45 et 75 F (7 et 24 C). L'infection des arbres vivants et des tronçons de tige frais coupés se réalise, dans des conditions optimales, pendant des périodes pluvieuses et lorsque la température moyenne journalière se tient entre 45 et 55 F (7 et 13 C). Selon les circonstances ambiantes, la prédisposition des surfaces blessées se modifie: elle s'abaisse rapidement et d'une manière irréversible après quelques jours de température élèves pendant l'été. Au dessus de 60 F (15 C), les champignons tels que le *Peniophora cinerea* (Pers. ex Fr.) Cooke, *Alternaria tenuis* Auct. et *Ceratocystis piceae* (Münch) Bakshi, exercent une concurrence serré pour occuper la surface exposée des blessures et, de ce fait, constituent le principal facteur qui restreint la période de sensibilité. Il semblerait que la suprématie du *S. sanguinolentum* à la surface des blessures fraîches serait due à la relative incapacité des organismes compétiteurs de tolérer certaines propriétés du substrat, surtout à de basses températures. Par ailleurs, la concurrence des saprophytes, qui élimine le pathogène, s'intensifie lorsque l'on altère le substrat soit en le baignant avec une solution d'extrait de malt, soit en excisant les parties blessées. La mort naturelle d'une branche ou du tronc produit le même effet.

Conditions influencing infection of artificially injured balsam fir (*Abies balsamea* (L.) Mill.) by *Stereum sanguinolentum* (Alb. & Schw. ex Fr.) Fr. were studied over a 3-year period in Quebec. Optimal conditions for spore dispersal and germination occurred during extended periods of high relative humidity and when mean daily temperatures were between 45 and 75 F (7 and 24 C). Optimal conditions for infection of both decapitated living trees and freshly excised stem sections occurred during periods of rain and when mean daily temperatures were between 45 and 55 F (7 and 13 C). Susceptibility of wound surfaces to *S. sanguinolentum* varied under different environmental conditions; it fell off rapidly, and irreversibly, after a few days of exposure to high summer temperatures. Intense competition for the wound surface at temperatures above 60 F (15.5 C) by such fungi as *Peniophora cinerea* (Pers. ex Fr.) Cooke, *Alternaria tenuis* auct. sensu Wiltshire, and *Ceratocystis piceae* (Münch) Bakshi was the main factor limiting the period of susceptibility. Experimental evidence suggests that dominance by *S. sanguinolentum* of the fresh-wound surface is due to the relative inability of competitors to tolerate certain substrate properties, particularly at low temperatures. Modification of the substrate, either by addition of malt extract solution, or by excision of the injured part (or by natural death) favored saprophytic competition which suppressed the pathogen.

Introduction

Recent studies (Davidson and Etheridge 1963) have shown that *Stereum sanguinolentum* (Alb. & Schw. ex Fr.) Fr. is a colonizer of fresh wounds of balsam fir, and it is through these that the fungus becomes established in the heartwood. The earlier belief that *S. sanguinolentum* entered the heartwood of balsam fir mainly through dead branch stubs (Kaufert 1935; McCallum 1928) probably resulted from the incorrect assumption that branches remain susceptible to colonization for an extended period after death or injury.

The importance of freshly exposed wound surfaces for primary-colonizing fungi, and the relatively short period of susceptibility of such substrates are becoming increasingly evident from studies on the ecology of stump infection by *Fomes annosus* (Fr.) Karst (Rishbeth 1951; Yde-Andersen 1962; Cobb and Schmidt 1964). Colonization of the stump surface by fungal competitors has been suggested as the main factor in limiting the infection period of *F. annosus* (Rishbeth 1951; Boyce Jr. 1963; Ginns Jr. 1968, personal communication). This has already been considered as a critical factor in the successful establishment of *S. sanguinolentum* via wound surfaces in balsam fir (Etheridge 1962, 1965).

Previous studies on fungi associated with *S. sanguinolentum* in balsam fir have been mainly concerned with species isolated from already established heart rot infections and there is

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little information on the kind, frequency, and interactions of the species involved in the initial colonization of the wound surface. The studies reported here were undertaken to define the conditions for infection of balsam fir by *S. sanguinolentum* in certain locations in Quebec, by determining the principal fungal and bacterial competitors of this fungus, the relative importance of environmental factors in limiting their occurrence, and the effect of these factors on the period of host susceptibility. Complementary studies were undertaken to determine the significance of inoculum and substrate factors in the infection process.

Materials and Methods

The principal study area was in a 60- to 70-year-old balsam fir stand at Duchesnay, approximately 20 mi northwest of Quebec City. Surveys had revealed that red heart rot caused by *S. sanguinolentum* together with its sporophores was of average abundance in the area. Other experimental areas were located in a 50-year-old balsam fir/spruce stand (fir: 20–30%; spruce: 60–75%) at Lac Jaune, about 3 mi from the main study area, and in a 30- to 35-year-old pure balsam fir stand located in the southern section of Laurentide Park, about 30 mi north of Quebec City.

Maximum and minimum temperatures, relative humidity, and precipitation were measured daily in the experimental area according to standard meteorological procedures, from 15 May to 15 September. Weather data for the rest of the experimental period were obtained from the records of the Government of Quebec meteorological station at Ancienne Lorette, 12 mi to the southeast.

Spore Dispersal and Germination

Estimates of spore discharge in the field were made by installing microscope slides, covered with a thin film of vaseline, about 1 in. below three to four naturally occurring sporophores of *S. sanguinolentum*. Every 24 hours for the period ending 9 to 10 A.M., the spores occurring on three transits across the top, middle, and bottom of the slide were counted with a low power microscope objective; the result was multiplied by an appropriate factor to obtain an estimate of the number of spores deposited over the slide surface.

In the study of spore discharge at various temperatures, microscope slides were placed on moist cotton under comparable portions of sporophores stuck to the lid of Petri dishes. Samples from each of three sporophores were used at each temperature. The chambers were then placed in polyethylene bags and incubated at appropriate temperatures over the range 35 F to 80 F for 24 h. Spore discharge was then estimated visually by the relative density of the spore deposits on the slide, according to an arbitrary scale, e.g., 1 (low) to 5 (high).

The criterion for spore germination was occurrence of a distinct germ tube after 24 h. Aqueous spore suspensions at density of 120 to 360 spores/mm^2 were dispensed on films of water agar (washed according to the method of Robins 1939), either directly on microscope slides, or on samples of host tissues, and incubated in moist chambers. Counts were based on three replicates, each of 100 spores. Germination at different temperatures was determined over the range 37 F to 83 F.

Preparation of Artificial Infection Courts and Methods of Microbiological Sampling

Living trees—Every 1–2 weeks during spring and summer of 1963 and 1964, six to eight balsam fir trees, 30 to 40 years old and 2–4 in. diameter breast height (d.b.h.), were "topped" about 8 ft from the ground; the saw cut was made several inches above the first whorl of green branches to avoid killing the tree. Five to 6 months after being injured, an 8-in. section of stem was cut from the remaining top of each tree, placed immediately in a polyethylene bag, and, within 4 hours, delivered to the laboratory. The sections were then swabbed with 95% ethanol, split longitudinally through the center, and eight small cubes of wood (approx. 0.5 cm^3) were removed aseptically from the heartwood and the sapwood at a distance of $\frac{1}{2}$ and 3 in. from the top surface. The samples of wood were placed on 2% malt agar slants in test tubes, and incubated at room temperature for 12–15 days.

Excised stem sections—Infection traps consisting of freshly cut sections of balsam fir stems, 4 in. long, unless otherwise stated, and 2–4 in. in diameter, were placed, usually in replicates of four, on tables 4 ft from the ground (Fig. 1). Actively sporulating fruit bodies of *S. sanguinolentum* were installed about 1 ft above the tables. Preliminary experiments indicated that an exposure time of 1–2 weeks was necessary to obtain a critical evaluation of temperature and precipitation effects on competition between initial colonizers of the wound surface. After exposure, the traps were enclosed in paper bags and incubated for 3 weeks at 60 F to permit the penetration of *S. sanguinolentum* to a suitable depth for sampling. The traps were then swabbed with 95% ethanol and split through the center four times to expose eight interior radial surfaces. Two isolations on 2% malt agar slants, one from the heartwood and one from the sapwood, were made from each radial surface (16 in all) and $1\frac{1}{4}$ in. from the exposed surface of the trap. Slants were examined after 12–15 days incubation at room temperature.

Control isolations were made, at the time of felling, from three sections of each of the trees which supplied material for the traps to determine the presence or absence of an indigenous microflora, as described above. Similarly, control isolations were made from the injured part of the living trees.

Factors Affecting Dispersal and Germination of Spores of *Stereum sanguinolentum*

Sporophore Production and Spore Discharge

Periodic observations in the Duchesnay study area since 1961 have revealed that fructifications of the fungus on slash appear during the first half of July of the third year after felling.

PLATE I

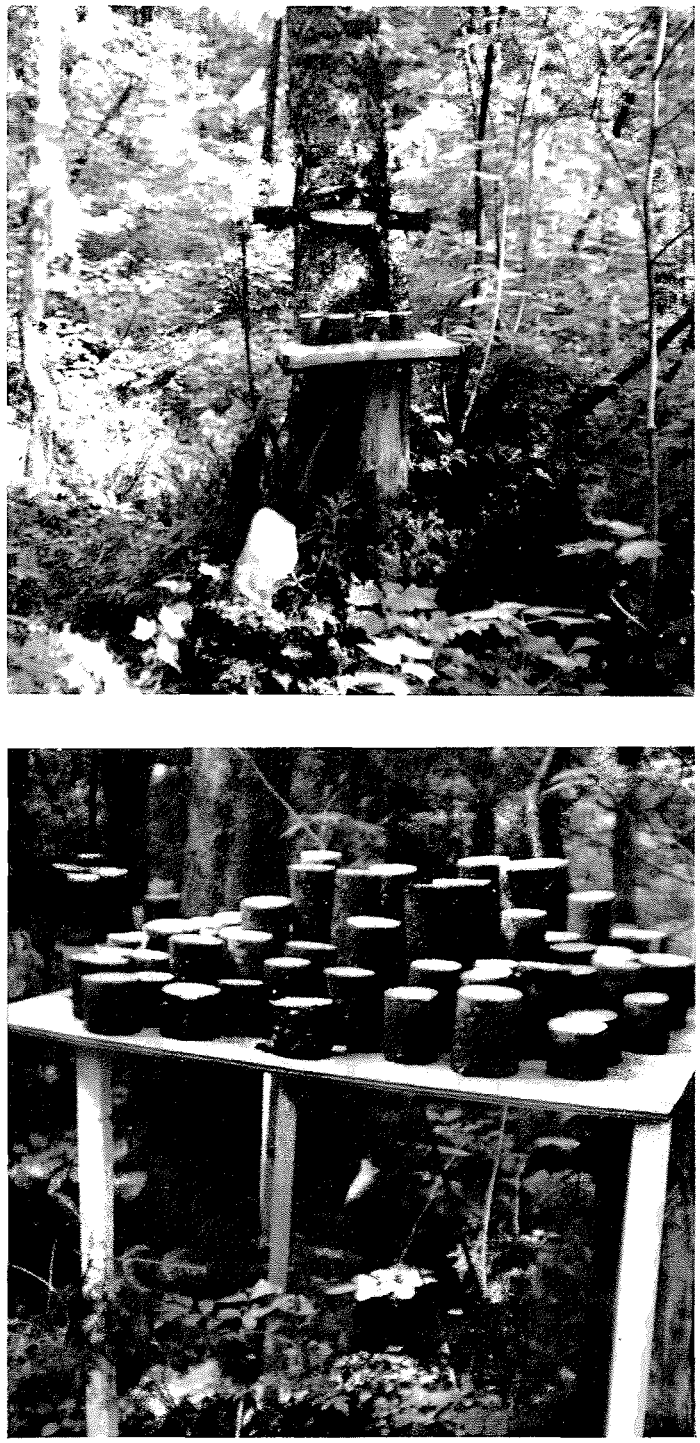


FIG. 1. Method of studying colonization of freshly cut balsam fir stem sections by microorganisms in the forest: (upper) a table of five standard, 4-in. stem sections exposed to natural inoculum showing position of sporophore of *S. sanguinolentum* installed on a board 1 ft above the table; (lower) sections of assorted sizes on a table in a study of the succession of microorganisms.

Spores were liberated during favorable conditions from July until the onset of freezing temperatures in October or November and spore liberation was resumed during the first week of April when temperatures were above freezing and there was an abundance of moisture from melting snow. The release of viable spores continued periodically from sporophores produced the previous year until after the appearance of the new crop of sporophores in July.

Spore-trapping surveys conducted in the forest at Duchesnay and in the arboretum at Quebec revealed that liberation of viable spores of *S. sanguinolentum* may occur from early spring to late fall, when conditions of 100% relative humidity prevail over several hours, especially during and immediately after periods of rain, and when mean daily temperatures are above about 40 F (Figs. 2 and 4). A substantial increase in spore discharge occurred on 4 and 12 October (Fig. 2), both dates being at the end of an extended rainy spell of 4 to 5 days. Generally, maximum liberation of spores was more often associated with several small rainfalls spread over 2 or more days than with a single, heavy downpour (Fig. 4).

Spore discharge under 100% relative humidity conditions in the laboratory was found to vary with temperature (Fig. 3, upper right). Discharge did not occur below about 35 F and above 78 F; the range of maximum discharge was between 50 and 70 F.

Germination

Basidiospores, at a concentration of 120 spores/mm^2 on a 2% water agar in a saturated atmosphere, showed 100% germination after 24 h at room temperature (72–73 F). Germination fell to 91% when the concentration was increased to 360 spores/mm^2 of agar surface. Concentrations of spores were kept within these two density values in subsequent tests. The factor which suppressed germination at the higher spore concentrations was found to be a specific, heat-unstable, water-soluble inhibitor associated with the spores. There was no evidence of the inhibitor affecting germination except under these unnatural conditions.

The maximum percentage of spore germination occurred at 70 F (Fig. 3); no germination occurred below 40 F or above 80 F. In contrast, no reduction in germination was evident between 70 and 50 F when the agar film was placed on fresh balsam fir heartwood and sapwood (Table I). However, in the latter case considerable reduction occurred in germ tube development at the lower temperature.

Germination tests were made also with spores from sporophores that had been kept at room temperature, and at 0 F for periods of up to 5

TABLE I

Percentage of germination and germ tube development of *Stereum sanguinolentum* and *Ceratocystis piceae* after 24 hours on water-agar films in contact with fresh and air-dried balsam fir heartwood and sapwood at temperatures of 50 and 70 F

Treatment	Percentage of germination		Germ tube length, μ	
	S. sanguinolentum	*C. piceae*	*S. sanguinolentum*	*C. piceae*
Fresh heartwood				
50 F	98	4 (25)*	13–117	3–44
70 F	100	100	26–117	65–208
Fresh sapwood				
50 F	100	5 (40)*	13–104	10
70 F	100	100	78–260	68–169
Air-dried heartwood				
50 F	98 (37)*	0 (55)*	12–32	—
70 F	100	100	13–273	35–130
Air-dried sapwood				
50 F	89	0	10–35	—
70 F	100	100	260–312	26–195
Moistened filter paper				
70–74 F	88	100	156–325	110–206

NOTE: A minimum of 100 spores were counted for each determination except for values marked with an asterisk (*), which are based on the number of spores shown in parentheses. Basidiospores of *S. sanguinolentum* were obtained from two fruit-bodies; conidia of *Ceratocystis piceae* were obtained from a culture grown on 2% malt extract agar.

to 6 months, in a dry condition. Storage for this length of time, at the test temperatures, appeared to have no appreciable effect on spore viability.

Spore germination tests were performed from time to time, usually weekly, with samples of basidiospores collected on water agar during the spore trapping surveys to determine whether seasonal variations occurred in viability; there was no evidence of any such variability.

Factors Affecting the Infection Process of *Stereum sanguinolentum*

Exposure of Fresh Wound Surfaces

(i) Natural Infection

Experiments with excised stem sections conducted during snow-free periods from April 1965 to June 1966 at Duchesnay, were designed, *inter alia*, to study the effect of exposure to forest air on susceptibility of wound surfaces to airborne spores of *S. sanguinolentum* (Fig. 1). A modification of the exposure treatment was attempted to study the influence of air on the susceptibility of the wound surface by excluding airborne spores for periods of varying duration. This is referred to as "conditioning". The treatment procedures were as follows.

Treatment A—Stem sections 4 in. in length were exposed on tables, 4 ft from the ground, to infection by airborne spores of *S. sanguinolentum* for 1 week (Fig. 4).

Treatment B—Stem sections 6 in. in length were placed on a wire grid raised slightly above the surface of the table so that only the top surface of the trap was exposed to the direct impingement of airborne spores. After 1 week, a 2-in. disk was removed from the top of the

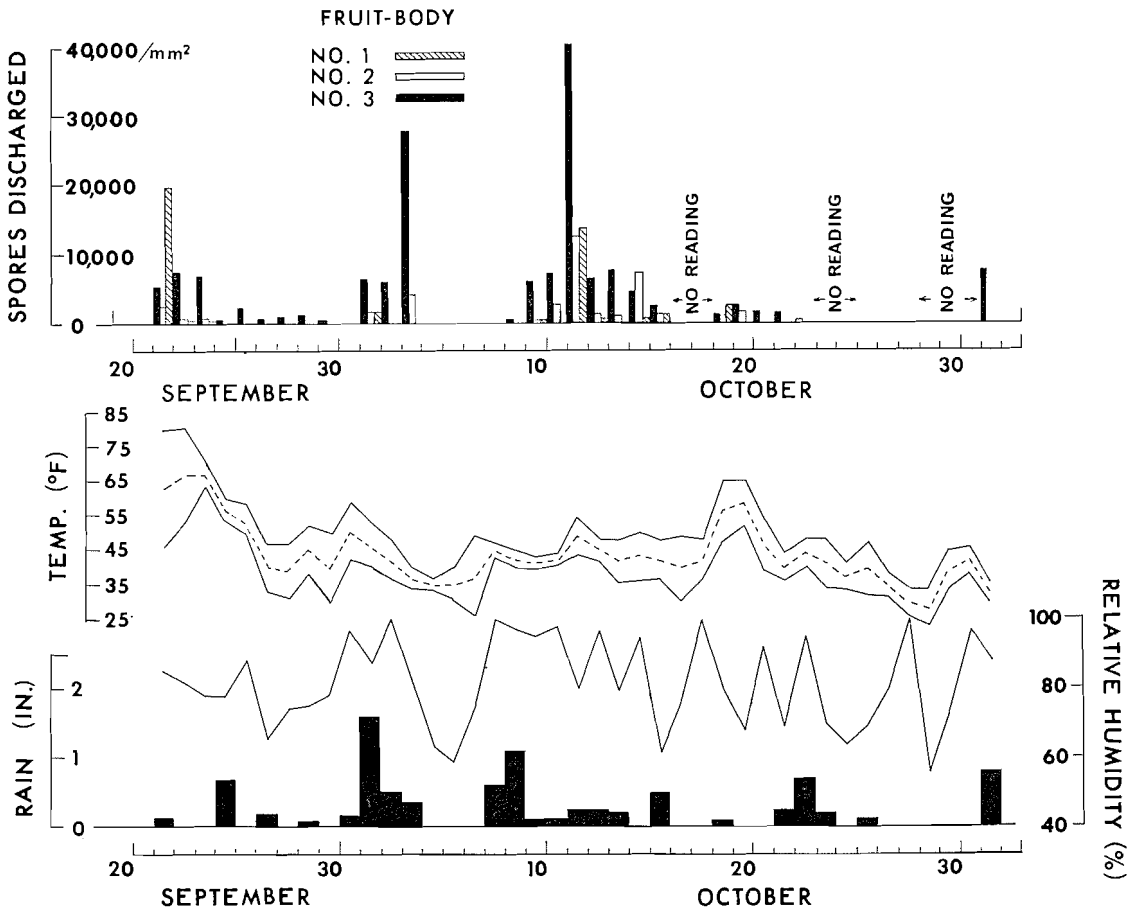


FIG. 2. Spore discharge of *S. sanguinolentum* in relation to maximum, mean, minimum temperature, relative humidity, and precipitation at Ste-Foy, Quebec.

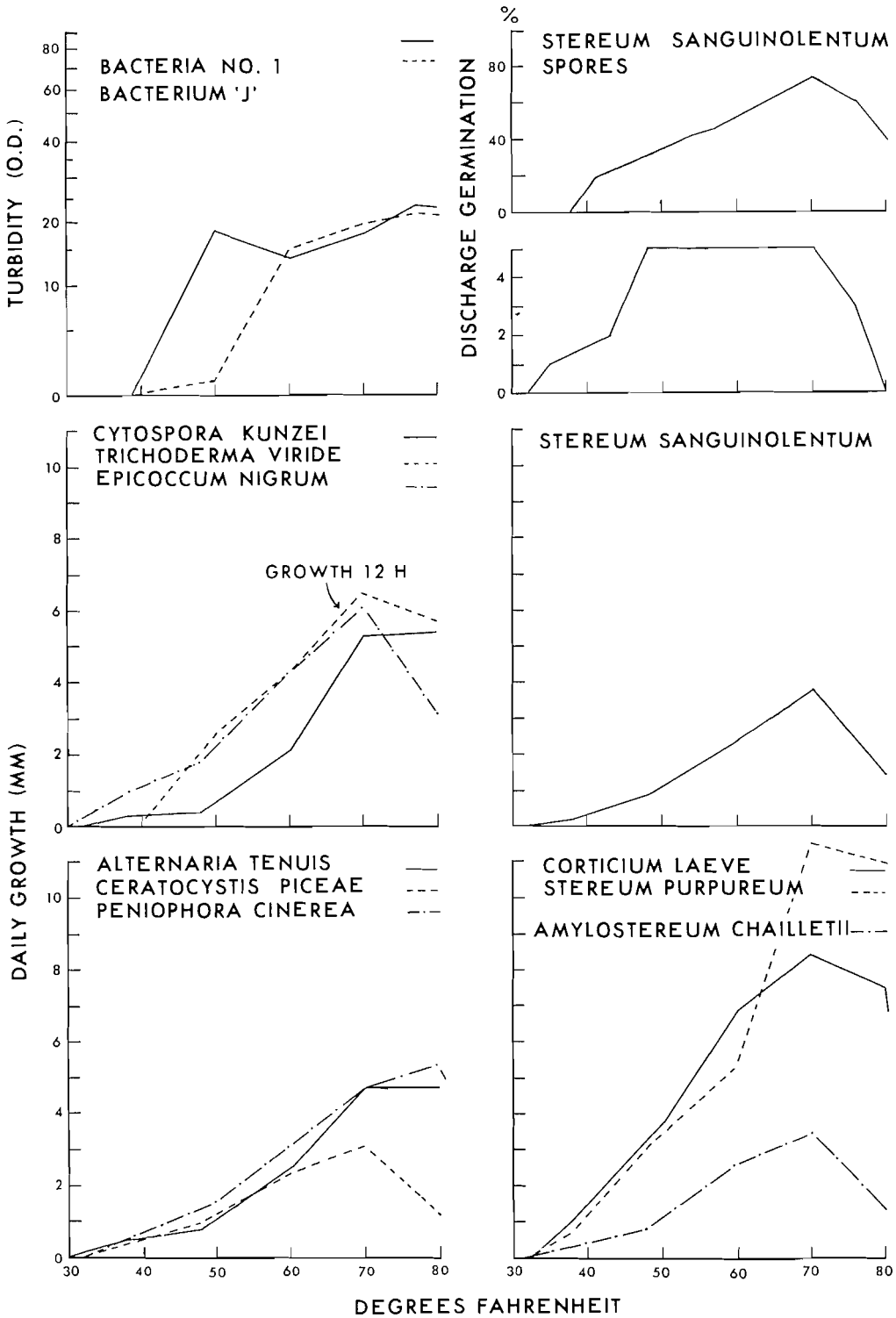


FIG. 3. Temperature relations of *S. sanguinolentum* and selected members of the microflora of freshly exposed balsam fir stems.

sections (to eliminate all chances of infection from that end) and the remaining 4-in. section was turned over; the bottom surface was then exposed for 1 week to infection by airborne spores (Fig. 6).

Treatment C—Stem sections 8 in. in length were placed on a table equipped with a wire grid, as for treatment B, and conditioned for 2 weeks. A disk of 4 in. was removed from the top end of the section before turning it over to expose the bottom surface for 1 week to infection by airborne spores (Fig. 7).

Treatment D—As for treatment C, except that no fruiting body of *S. sanguinolentum* was installed above the traps.

Treatment E—Stem sections 4 in. in length were exposed for *2 weeks* to infection by airborne spores under the same conditions as for treatment A (Fig. 5).

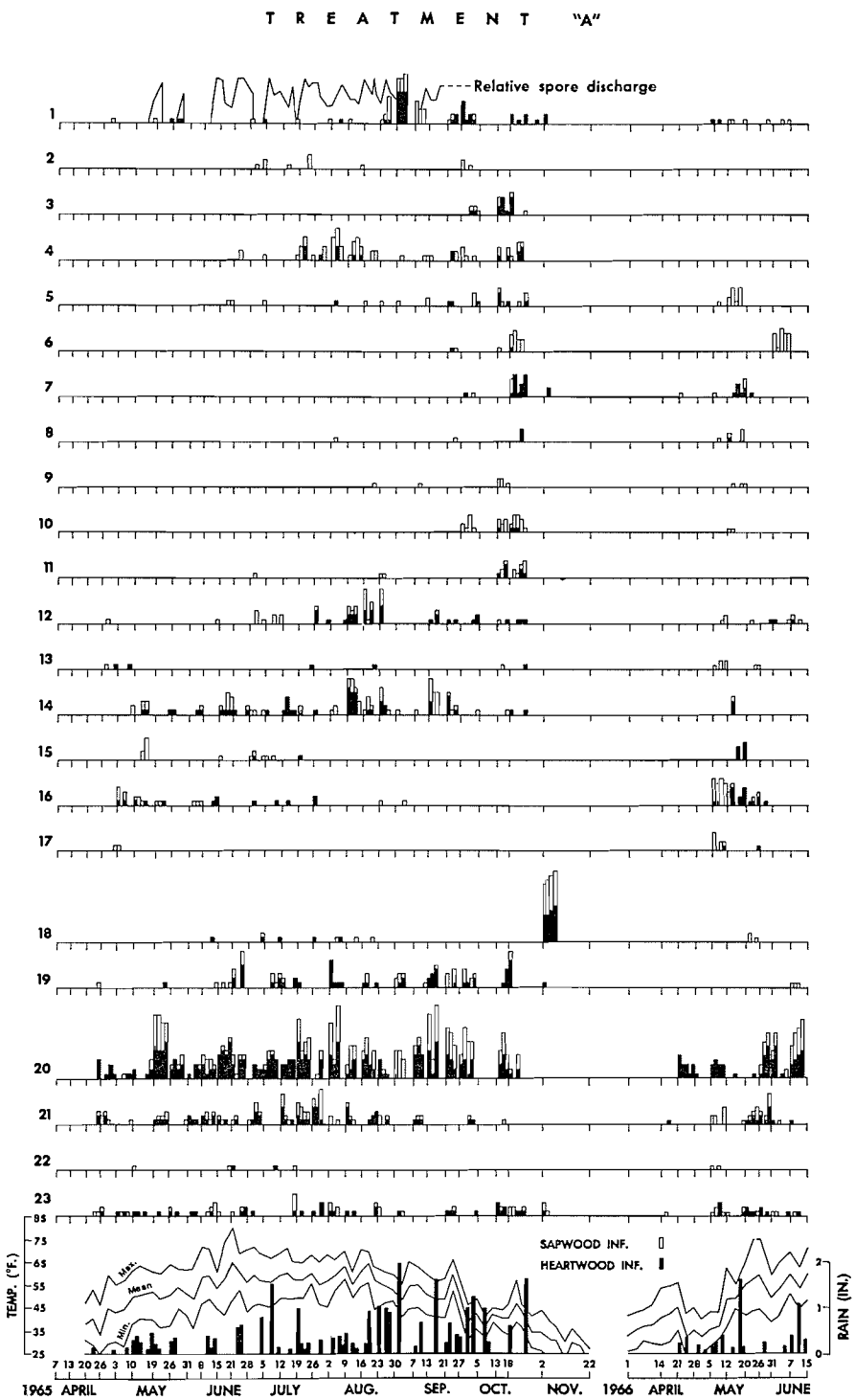
The traps were exposed on the tables, in treatment groups of four or five, at intervals of 6–7 days (Fig. 1). All traps for a given exposure period came from the same stem. In every case, incubation was for 3 weeks at 60 F after the treatments.

The histograms of Figs. 4 to 7 show the kind and frequency of isolation of species colonizing balsam fir stem sections throughout the period of study, for exposure treatments A, E, B, and C, respectively. Figures 4 and 5 (treatments A and E) show the pattern of colonization after 1 and 2 weeks of exposure, respectively. The effect of the second week of exposure on *S. sanguinolentum* was negligible, indicating that infections had occurred during the first week. The second week, however, resulted in an increase in the frequencies of *Ceratocystis piceae* (Münch) Bakshi, *Cytospora* sp., and bacterium "J". The frequency of the six fungi, *Peniophora cinerea* (Fr.) Cke., *Corticium laeve* Pers. ex Fr., *Corticium* sp., *Trechispora* sp., *Penicillium* sp., and *Alternaria tenuis* auct. sensu Wiltshire was reduced by varying degrees as a result of the additional exposure. *Peniophora cinerea* was most markedly suppressed by the extended exposure period and showed decreased frequencies during periods when *Ceratocystis piceae* and bacterium "J" were showing an increase, indicating that it was probably replaced by the latter two species.

Figures 6 and 7 show the frequencies and patterns of colonizing species after 1 and 2 weeks of conditioning, respectively, followed by a week of exposure to infection by airborne spores (treatments B and C). By comparison with Figs. 4 and 5, it can be seen that all of the species of decay fungi, with the exception of *Amylostereum chailletii* (Pers. ex Fr.) Boid., as well as most of the other colonizing species, were drastically suppressed by the exclusion of airborne spores during the conditioning period. Infection by *Stereum sanguinolentum*, in particular, shows a marked reduction as a result of the conditioning when compared to the relatively high levels of infection obtained for the same exposure periods in traps without this pretreatment (treatments A and E).

Ceratocystis piceae and bacterium "J" colonized the "conditioned" surfaces, and continued to colonize with increasing intensity with an

FIGS. 4 to 8. The frequency of microorganisms isolated from freshly cut balsam fir stems after various exposure treatments in relation to maximum, mean, minimum temperatures and precipitation for the period studied. The solid histograms refer to heartwood infections, empty histograms to sapwood infections. A histogram of maximum height (8 units) indicates that the particular microorganism was obtained from each of eight isolations attempted from the heartwood or sapwood of an individual stem section. A square histogram (1 unit) indicates a single isolation of a particular microorganism from an individual section. Exposure periods (6 to 7 days) are indicated by divisions on the horizontal time scale. For any period, the histograms give frequencies for each section exposed. The numbers at lefthand margin refer to the following microorganisms: (1) *Stereum sanguinolentum*, (2) *Amylostereum chailletii*, (3) *Stereum purpureum*, (4) *Peniophora cinerea*, (5) *Peniophora gigantea*, (6) *Polyporus adustus*, (7) *Corticium laeve*, (8) *Corticium* sp.[1], (9) *Corticium* sp.[2], (10) *Trechispora* sp., (11) unknown and miscellaneous basidiomycetes, (12) *Alternaria tenuis*, (13) *Epicoccum nigrum*, (14) *Ceratocystis piceae*, (15) *Ceratocystis* sp., (16) *Cytospora kunzei*, (17) *Cytospora* sp. (M-3), (18) *Trichoderma viride*, (19) bacterium "J" (yellow-pigmented), (20) bacteria "1", (21) *Penicillium* spp., (22) *Thyronectria* sp., (23) unknown and miscellaneous non-basidiomycetous fungi. Fig. 4. Treatment A: traps exposed 1 week with viable sporophore of *Stereum sanguinolentum* installed over table. Curve (upper diagram) shows relative availability of basidiospores of *S. sanguinolentum* during the exposure periods. Fig .5. Treatment E: stem sections exposed 2 weeks with viable sporophore of *S. sanguinolentum* installed over table. Figs. 6, 7. Treatment B: stem sections conditioned 1 week (see text), then exposed 1 week. Viable sporophore of *S. sanguinolentum* installed over table. Treatment C: stem sections conditioned 2 weeks, then exposed 1 week. Viable sporophore of *S. sanguinolentum* installed over table. Fig. 8. Comparative frequency of *S. sanguinolentum* isolated from stem sections subjected to the different treatments for the period studied.


T R E A T M E N T "A"
Relative spore discharge
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TEMP. (°F.)
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SAPWOOD INF.
HEARTWOOD INF.
RAIN (IN.)
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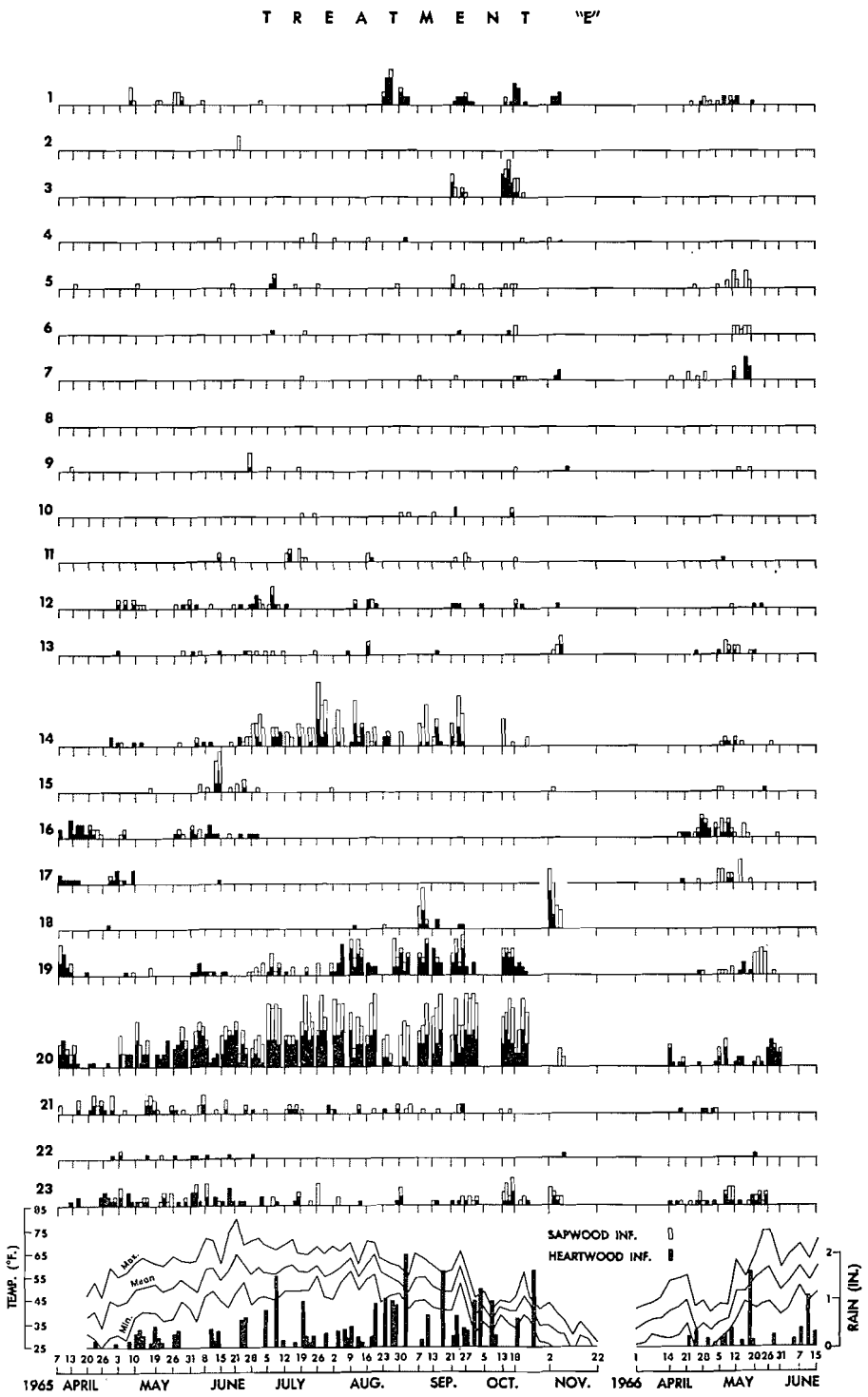
1 14 21 28 5 12 20 26 31 7 15
1965 APRIL MAY JUNE JULY AUG. SEP. OCT. NOV.
1966 APRIL MAY JUNE

FIG. 4.


TREATMENT "E"
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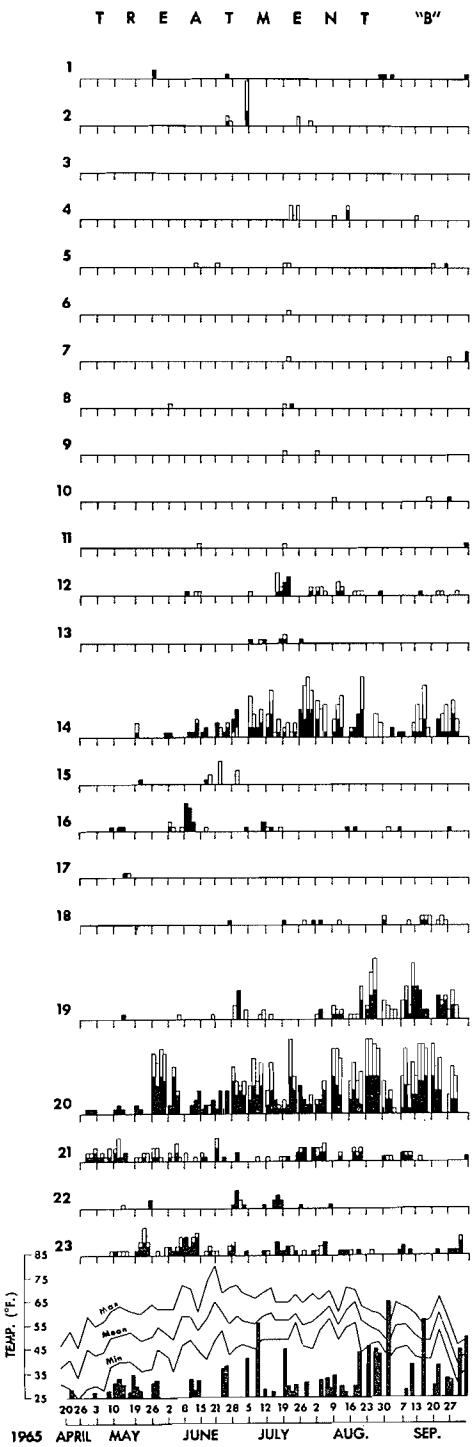
1965 APRIL MAY JUNE JULY AUG. SEP. OCT. NOV.
1966 APRIL MAY JUNE

FIG. 5.


TREATMENT "B"
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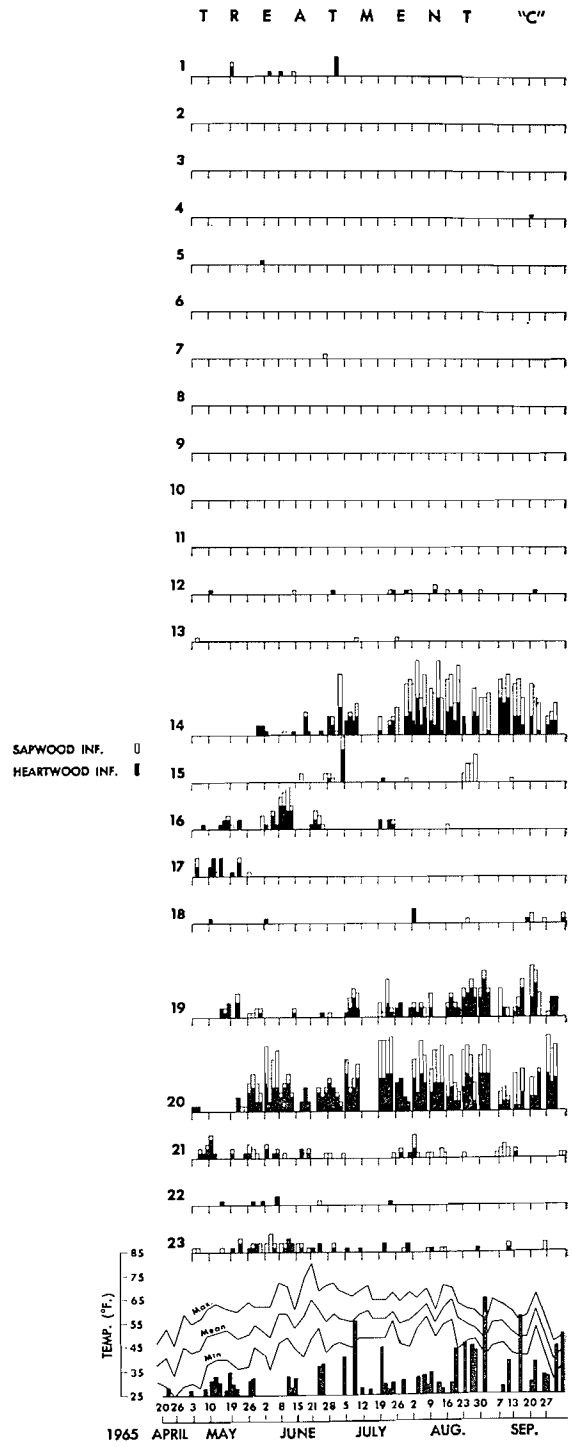
1965 APRIL MAY JUNE JULY AUG. SEP.

FIG. 6.


SAPWOOD INF.
HEARTWOOD INF.
TREATMENT "C"
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TEMP. (°F.)
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1965 APRIL MAY JUNE JULY AUG. SEP.

FIG. 7.

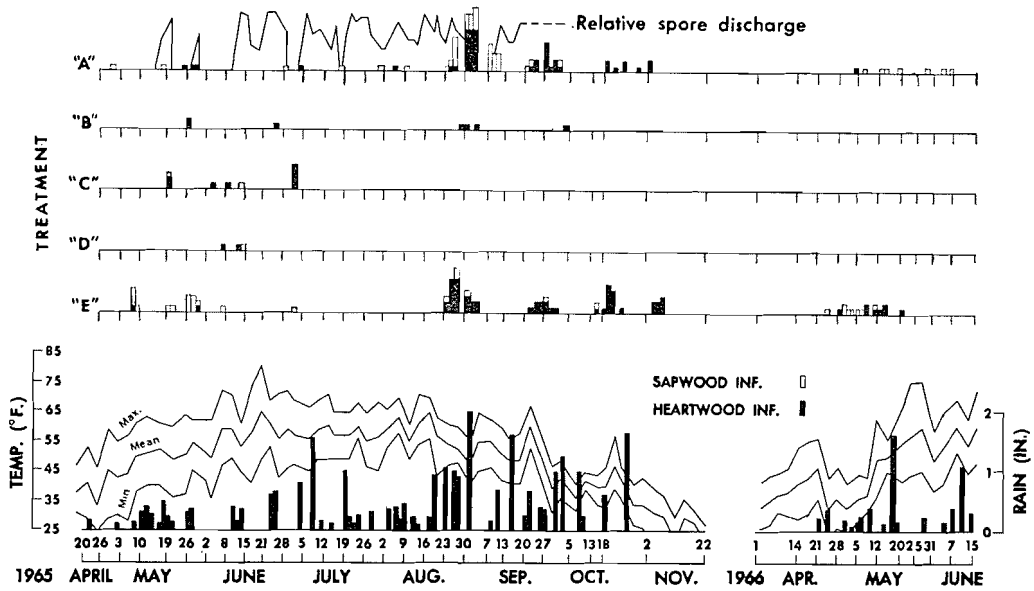

FIG. 8.

increase in exposure time to airborne spores (Figs. 4 and 5). A reduction in the frequency of *Alternaria tenuis* and *Peniophora cinerea*, similar to that which resulted from extended exposure to airborne spores, occurred as a result of the additional week of conditioning, supporting the earlier observation that *Peniophora cinerea* was being replaced by *Ceratocystis piceae* and bacterium "J".

Inoculum Availability

Limited information on the effect of the proximity of sporophores on infection was obtained during the course of the "trapping" experiments. A comparison was made of the frequency of *S. sanguinolentum* recovered from traps which had been exposed under similar conditions in the forest, except that one set of traps was located about 1 ft from an actively sporulating fruiting body (treatment C), while the other was at least 10 ft from the nearest fruiting body (treatment D). The frequency of isolations of *S. sanguinolentum* recovered over a 2-month period was 10 from traps placed under the sporophore and only 3 from the traps with no sporophore present (Fig. 8).

(ii) Artificial Infection

The effect of various environmental factors on infection of balsam fir stem sections after artificial inoculation with basidiospores of *Stereum sanguinolentum* was investigated under controlled conditions in the forest and in the laboratory. Fifty-one stem sections, 4 in. long, were cut from a single tree on 23 August 1965 and treated as follows:

(1) 16 sections were immediately exposed to forest air by the method used in the natural infection experiments;

(2) 16 sections were immediately exposed to forest air under bell jars which had been placed on a raised wire grid provided with several layers of loosely packed cheesecloth to filter the circulating air;

(3) 16 sections were placed in a cold room at −17 C immediately after dissection on the same day as felling;

(4) 3 sections coming from the base, middle, and top of the stem were used to estimate the moisture content of the tree and to determine the presence or absence of an indigenous microflora at the time of felling.

Two weeks after this treatment (7 September 1965), the stem sections were assembled in the laboratory and their moisture content adjusted to a comparable excess level by allowing each section to soak in 100 ml sterile distilled water for several hours. The stem sections were then inoculated at two points (1 in. apart), at the

TABLE II

Recovery of microorganisms from balsam fir stem sections exposed to various pre- and post-inoculation treatments†

	Treatment																											Totals						
	Air and rain in forest									Filtered air in forest										Unexposed, kept at −17 C															
	50 F				70 F					50 F				70 F					50 F				70 F											
	Wet		Dry		Wet		Dry			Wet		Dry		Wet		Dry			Wet		Dry		Wet		Dry									
	P	M	P	M	P	M	P	M	T	P	M	P	M	P	M	P	M	T	P	M	P	M	P	M	P	M	T	50 F	70 F	Wet	Dry	P	M	Grand total
	*Stereum sanguinolentum**																																	
H‡	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	2	1	0	3	0	0	0	0	0	4	6	0	2	4	6	0	6
S	0	0	0	0	0	0	1§	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1
T									1									2									4	6	1	2	5	7	0	7
	Other basidiomycetes																																	
H	0	0	0	4	1	0	1	0	6	1	1	0	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	7	2	3	6	3	6	9
S	0	0	0	4	1	0	0	1	6	0	2	1	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	7	2	3	6	2	7	9
T									12									6									0	14	4	6	12	5	13	18
	Ceratocystis piceae																																	
H	0	0	0	0	0	1	4	4	9	0	3	0	4	6	6	5	2	26	1	11	3	9	2	6	7	5	44	31	48	36	43	28	51	79
S	0	2	6	7	1	4	10	10	40	0	1	4	10	5	2	10	11	43	3	1	3	9	3	9	8	6	42	46	79	31	94	53	72	125
T									49									69									86	77	127	67	137	81	123	204
	Trichoderma viride																																	
H	1	0	0	0	0	2	0	4	7	1	2	0	4	6	4	5	9	31	0	0	4	2	3	1	7	12	29	14	53	20	47	27	40	67
S	4	0	2	0	1	0	1	1	9	11	0	7	3	2	1	8	11	43	0	0	8	7	7	4	12	12	50	42	60	30	72	63	39	102
T									16									74									79	56	113	50	119	90	79	169
	Bacterium "J"																																	
H	7	6	7	6	5	0	2	3	36	2	2	0	0	2	1	0	1	8	1	1	0	0	0	0	0	0	2	32	14	27	19	26	20	46
S	1	0	3	0	1	1	0	0	6	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	4	3	3	4	6	1	7
T									42									9									2	36	17	30	23	32	21	53
	Bacteria "I"‖																																	
H	11	12	12	12	12	12	7	10	88	11	11	12	11	12	11	11	12	91	12	11	11	11	12	12	11	12	92	137	134	139	132	134	137	271
S	11	11	12	7	11	10	6	11	79	10	12	11	12	12	12	12	12	93	12	12	12	11	12	10	12	12	93	133	132	135	130	133	132	265
T									167									184									185	270	266	274	262	267	269	536
	Other non-basidiomycetes																																	
H	1	0	0	1	0	1	1	0	4	0	0	0	1	2	0	0	0	3	3	0	1	0	1	1	0	0	6	7	6	9	4	9	4	13
S	0	1	1	0	0	0	0	1	3	0	0	0	0	0	1	1	0	2	0	0	0	0	1	1	0	0	2	2	5	4	3	3	4	7
T									7									5									8	9	11	13	7	12	8	20

*The organisms were recovered on the following sample basis: 24 isolations (12 heartwood, 12 sapwood) from four stem sections receiving each treatment.
†Exposure period = 23/8/65 to 7/9/65; constant temperatures of 50 and 70 F maintained during week after inoculation. Wet treatment = sterile distilled water added to stem sections at rate of 200 ml during first week to simulate 1 in. of rain. P = pure suspension of spores of *S. sanguinolentum*; M = mixed suspension of spores of *S. sanguinolentum* + *C. piceae* + miscellaneous microorganisms.
‡H = heartwood; S = sapwood; T = totals.
§Assumed to be natural infection.
‖Recovered also from heartwood (12%) and sapwood (5%) at time of felling.

juncture of the heartwood and sapwood, on one side of the top surface with 0.2 ml of a suspension of spores of *S. sanguinolentum* and on the other side with 0.2 ml of a mixed suspension of spores of *S. sanguinolentum*, *Ceratocystis picea*, and miscellaneous microorganisms. The suspension of miscellaneous microorganisms was obtained from collections of rainwater made in the forest during the previous 2 weeks. After inoculation, randomly selected samples from each of the three initial-treatment groups, were incubated at two temperatures (50 and 70 F) and with and without water amendments for 1 week, then for a further 3 weeks at 60 F. Infection was determined on the basis of six isolations (three heartwood, three sapwood) taken at a depth of $1\frac{1}{4}$ in. immediately below each of the paired inoculation points on the surface of each section. Four stem sections received each treatment. Further pertinent details and the results of the experiment are given in Table II.

The results generally support the conclusions drawn from the natural infection experiments, namely, that *S. sanguinolentum* is decidedly favored by fresh, uncolonized heartwood and by temperatures below 60 F, conditions which appeared to reduce competition by other organisms to a minimum. It is notable that on a fresh substrate ("unexposed" treatment), *Trichoderma viride* occurred almost three times as frequently at 70 F as at 50 F and almost twice as frequently in the sapwood as in the heartwood and appeared to be the main reason for the total exclusion of *S. sanguinolentum* in these situations. It is notable also that the frequency of isolation of bacterium "J" was appreciably greater in stem sections exposed to forest air and rain than in treatments where rain was excluded. The wetting-by-rain treatment was marked also by complete loss of susceptibility to the artificially introduced spores of *S. sanguinolentum* (the single infection recorded being regarded as a natural one) which produced infections only with treatments that appeared to suppress bacterium "J". The moisture amendments during the initial week after inoculation had a depressing effect on most of the colonizing fungi and did not appear to be selective for any particular organism.

Moisture Content of Wood

It was considered important to determine whether changes in moisture content occurred during exposure which could have a significant effect on the infection process. Consequently, determinations were made of the moisture content (oven-dry weight basis) of representative balsam fir stem sections, after exposures of increasing duration, during 1965 (Table III).

Variations occurred between the initial moisture content determinations for each series of exposures, ranging from 99% for samples installed on 23 August 1965 to 179% for samples installed on 2 August 1965, which reflected individual differences in moisture content of the sample trees at time of felling. With the exception of the series of 26 July 1965 which showed marked fluctuations in moisture content during the 3-week exposure period, only minor fluctua-

TABLE III

Effect of exposure (forest) and incubation (laboratory) treatments on the moisture content of representative balsam fir stem sections, for specific trapping periods in 1965 (given as percentage of mean moisture content with the determination date in parentheses)

Initial (fresh-cut)	After 1 week exposure	After 2 weeks exposure	After 3 weeks exposure	After 1 week exposure and 3 weeks incubation
134.8 (26/7/65)	92.2 (2/8/65)	139.2 (9/8/65)	81.9 (16/8/65)	93.2 (23/8/65)
179.2 (2/8/65)	136.7 (9/8/65)	142.2 (16/8/65)	158.4 (23/8/65)	84.4 (30/8/65)
107.7 (9/8/65)	95.0 (16/8/65)	113.4 (23/8/65)	110.9 (30/8/65)	57.4 (6/9/65)
147.8 (16/8/65)	131.8 (23/8/65)	137.9 (30/8/65)	148.7 (6/9/65)	86.8 (13/9/65)
99.4 (23/8/65)	85.8 (30/8/65)	78.7 (6/9/65)	78.0 (13/9/65)	78.1 (20/9/65)
148.5 (30/8/65)	133.8 (6/9/65)	123.6 (13/9/65)	142.5 (21/9/65)	—
Averages				
136.2	112.5	122.5	120.1	80.0

NOTE: Moisture content expressed as percentage of oven dry weight: minimum of three replicates for "initial", four for "1 week", four for "2 weeks", one for "3 weeks", and one for "incubation".

tions were recorded for the other series and these represented a spread of values only slightly lower at the end of 3 weeks (78% to 158%) than occurred initially. Exposure at 60 F during the 3-week incubation period produced a more marked drop in moisture content and final values ranged from 57% to 93%.

Precipitation

Observations were also made on the effect of rain on the incidence of infection of decapitated, living balsam fir (Fig. 9) and excised stem sections (treatments A and E, Fig. 8) by *S. sanguinolentum*. Analysis of the data showed that infection of living trees, more clearly than that of the stem sections, is affected by rain at the time of wounding; therefore, results pertaining only to decapitated trees are presented. Generally, rain was relatively plentiful and well distributed over the study period so there was no clear example of the effect of extended drought periods on infection. Despite several examples of infection occurring in trees during short drought periods of 3 to 4 days duration after wounding, notably, on 25 April 1963 and 1 May 1964 (Fig. 9), such dry spells after wounding usually prevented infections by *S. sanguinolentum*. Almost 80% of the injury dates which resulted in infection by this fungus coincided with wet periods when rain fell on the day of the injury, or the next day. Rain falling on the day before the injuries were made appeared to have no effect on subsequent infection by *S. sanguinolentum*.

A critical comparison of the occurrence of rain at Duchesnay during the summer and fall of 1965 (Fig. 8) and the moisture march in stem sections exposed over the same period (Table III), failed to show a quantitative relationship between rainfall and the moisture fluctuations in the wood.

Temperature

The exposure period immediately after woundings which resulted in establishment of *S. sanguinolentum*, either in living trees or in excised stem sections, was characterized by mean daily temperatures within the range of 38 F to 60 F (Figs. 8 and 9). In contrast, a marked reduction in the frequency of infection can be observed as mean daily temperature values approached 60 F; for example, during June and July 1963, June 1964 (Fig. 9), and June, July, and the first half of August 1965 (Fig. 8). The marked contrast between the frequency of injured trees infected by *S. sanguinolentum* during April and

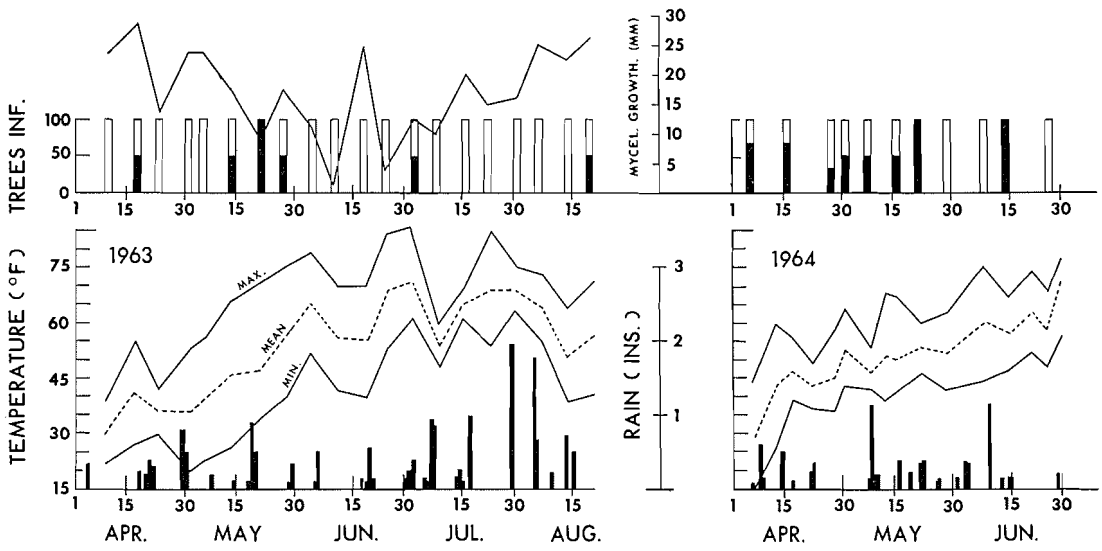

Fig. 9. Infection by *S. sanguinolentum* (upper diagram) of decapitated living balsam fir trees in relation to maximum, mean, minimum temperature and precipitation at Duchesnay, Quebec (lower diagram) and the suitability of the wood substrate of the injured trees to support mycelial growth of this fungus (curve, upper diagram).

May 1963 and these same months in 1964, appears to be due to the occurrence of suboptimal temperatures for this fungus for an extended period during the spring of 1963.

The effect of ambient temperatures on the kind and frequency of colonizing species associated with *S. sanguinolentum* in excised stem sections of balsam fir can be seen in Figs. 4 and 5. Species that were apparently favored by the same general range of temperatures as *Stereum sanguinolentum* were *Stereum purpureum* (Pers. ex Fr.) Fr., *Polyporus adustus* Willd. ex Fr., *Corticium laeve*, *Corticium* sp., *Trechispora* sp., *Ceratocystis* sp. (*Chalara* type), *Cytospora kunzei* Sacc., and *Cytospora* sp. With the exception of *Stereum purpureum*, which occurred late in the season, and *Cytospora kunzei*, which occurred in the spring, none of these fungi was sufficiently abundant to be regarded as a serious competitor. Species which occurred at temperatures above the maximum of the range for *Stereum sanguinolentum* (60 F), and which, therefore, could be of major importance in determining the failure of this fungus to colonize freshly exposed wound surfaces at the higher temperatures, were *Peniophora cinerea*, *Alternaria tenuis*, *Ceratocystis piceae*, and possibly bacterium "J". All of these species were isolated relatively infrequently from traps installed during the period 23 August to 7 September 1965, when maximum colonization by *Stereum sanguinolentum* was obtained.

For practical purposes the cardinal temperatures for the growth of a fungus in wood are approximately the same as for its growth on malt agar (Cartwright and Findlay 1946). This was confirmed by comparative studies of the rates of linear extension of *S. sanguinolentum* mycelia in balsam fir stems and on 2% malt agar under controlled conditions in the laboratory. Longitudinal penetration of wood by *S. sanguinolentum* at different temperatures was determined by installing freshly cut stem sections of balsam fir (about 3 ft long) in growth chambers maintained at 37 F, 48 F, 60 F, and 71 F, respectively. The base of each section was placed in a pan of water to maintain a uniform wood moisture content during the incubation period; the tops were covered with sheets of plastic fastened tightly around the bark. The sections were inoculated in duplicate, about 6 in. from the top, by inserting plugs of wood (1 in. in length) infected with *S. sanguinolentum* into holes drilled aseptically to appropriate depths in the sapwood and heartwood. The holes were then sealed with grafting wax. After 6 weeks, the sections were split longitudinally and isolations were attempted, at $\frac{1}{2}$-in. intervals, from directly below each point of inoculation.

Radial growth on 2% malt agar was determined for temperatures over the range of 37 F to 86 F. Two measurements at right angles to the diameters of duplicate colonies were made at the end of 7 and 14 days. The difference between the measurements which represented the growth during the second week was used to calculate the mean daily growth of the cultures.

Maximum and minimum growth rates of about 4 in. and $\frac{1}{3}$ in. per month at 71 F and 37 F, respectively, were obtained on both substrates. Therefore, the effect of temperatures on the growth of *S. sanguinolentum* and selected fungi were studied on 2% malt agar (Fig. 3). The temperature for optimal growth of most of the wound colonists of balsam fir was 70 F. However, five species, including three of the four suspected competitors of *S. sanguinolentum* at higher temperatures; namely, *Alternaria tenuis*, *Peniophora cinerea*, and bacterium "J" had higher optima, around 80 F, than the others. *Ceratocystis piceae* had about the same temperature requirements for mycelial growth as had *Stereum sanguinolentum*, which suggested that factors other than rate of colonization of wound surfaces, possibly inoculum availability, gave this fungus an advantage at temperatures above 60 F.

Comparative studies of *S. sanguinolentum* and *Ceratocystis piceae* were made in the laboratory on exposed as well as on previously unexposed heartwood and sapwood of balsam fir to determine the relative effect of high and low incubation temperatures on spore germination. The results presented in Table I show that basidiospores of *S. sanguinolentum* germinated equally well at 50 and 70 F after 24 hours, while germination of conidia of *C. piceae* was markedly arrested at the lower temperature. At these two temperatures neither sapwood nor heartwood appeared to have any differential effect on germination or germ tube development of the two fungi.

Substrate

Periodically, from 1963 to 1965, *Stereum sanguinolentum* and selected fungal competitors were grown on freshly cut stem sections of balsam fir under uniform temperature and moisture conditions to investigate possible variable growth-promoting or growth-retarding effects of the substrate. Tests were conducted with material from (i) different heights along an individual stem, (ii) the same heights of different trees, (iii) heartwood and sapwood areas of individual disks, and (iv) trees cut at different seasons of the year. Sections about $\frac{1}{2}$ in. thick were cut aseptically with a hand saw from appropriate samples of freshly decorticated balsam fir stems which had been kept frozen since the time of felling. The sections were placed immediately in sterile Petri dishes or other suitable vessels. Control material, to determine if the effects were of microbiological or chemical origin, was similarly prepared from adjacent parts of the stem and then autoclaved for $\frac{1}{2}$ h at 15 lb/sq. in. Before they were tested, the sections were soaked in sterile, distilled water for 2 to 3 h to obtain a uniform moisture content. Dialyzer paper which had been dry-sterilized with propylene oxide gas was placed on the surface of the wood after it was wetted first in sterile distilled water to ensure a good contact. Sections were inoculated along two radii with 5-mm plugs from malt agar cultures; the surface of the mycelial mat was placed in contact with the dialyzer paper. After inoculation, the cultures were placed in polythene bags and incubated at 23 C. Colony diameters were measured after 7 days with the aid of a low-power stereo microscope.

Over the 2-year period, during which tests on several dozen trees were carried out, there was some indication that *S. sanguinolentum* grew better on heartwood than on sapwood, and on heartwood from the upper stem than on heartwood from the base of trees, but individual variation was considerable and no consistent patterns of substrate susceptibility or resistance were revealed. Similar results were obtained in tests with autoclaved sister disks, which suggested that the observed variation was not due to the presence of an indigenous microflora. However, the sterilized material usually supported better mycelial growth, particularly for fungal competitors of *S. sanguinolentum*.

In addition, the possibility that variable growth-promoting or growth-retarding factors of the wood were important in regulating infection of balsam fir by *S. sanguinolentum* was investigated by carrying out similar tests on freshly cut disks taken from the stem adjacent to the wound surfaces of trees experimentally decapitated during 1963 and 1964. The average colony diameters of the fungus on these substrates for successive wounding dates is given in the upper diagram of Fig. 9. It appears that no quantitative relationship existed between substrate suitability to *S. sanguinolentum* and natural infection of the trees. It was unfortunate that the two injury dates, i.e., 10 June and 25 June, 1963, when almost complete growth inhibition by wood was obtained against *S. sanguinolentum*, coincided with otherwise unfavorable weather conditions which made further examination of the effects impossible.

Although control tests with autoclaved material indicated that microorganisms were probably not actively involved in causing the observed inhibitory effects, species of bacteria are known to be an important component of the inner heartwood of living balsam fir (Etheridge and Morin 1967), and as such could play a significant role in the infection process. In the present study, a bacterial complex (designated as bacteria "1") was made up of the organisms most frequently isolated from both the excised stem sections (Figs. 4 to 8) and the injured stems of living trees (Table V). This bacterial complex occurred at about the same frequency in the many control isolations made from the sample trees at the time of felling. However, under the wide range of conditions studied, there was no evidence of it acting in any way as a competitor of *S. sanguinolentum*. The relative growth rates (in 0.8% nutrient broth after 24 h), at different temperatures, for bacteria "1" is given in Fig. 3, where it can be seen that moderately high activity occurs over a range of 45 to 55 F. As noted earlier, this is optimal for infection of fresh wound surfaces by *S. sanguinolentum*. Moreover, pairing tests with *S. sanguinolentum* and members of bacteria "1" on 2% malt agar showed no evidence of antagonism between the colonies. Recent studies by R. J. Bourchier, Fredericton, N.B., have confirmed these observations (personal communication).

TABLE IV

Frequency of microorganisms isolated from balsam fir stem sections exposed (for periods of 1 month) in balsam fir stands of different composition and ages, between the dates shown

Organisms		Duchesnay, mature fir (70%)					Lac Jaune, immature fir (25%)					Laurentide Park, immature fir (90%)				
		1	2	3	4	T	1	2	3	4	T	1	2	3	4	T
Basidiomycetes																
Stereum sanguinolentum	H	0	0	0	0	0	0	0	1	0	1	0	0	3	1	4
	S	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Stereum purpureum	H	2	0	0	0	2	0	0	1	0	1	0	0	0	1	1
	S	1	1	0	0	2	0	8	0	0	8	0	0	0	0	0
Peniophora cinerea	H	0	0	0	0	0	1	1	0	0	2	0	0	0	0	0
	S	1	0	0	0	1	6	0	1	0	7	0	0	0	0	0
Peniophora gigantea	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Polyporus adustus	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Corticium laeve	H	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	S	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Trechispora sp.	H	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
	S	0	0	0	1	1	0	0	0	2	2	0	0	0	0	0
Unknown and miscellaneous	H	0	0	0	0	0	1	1	0	0	2	0	0	2	0	2
	S	0	0	1	1	2	0	0	0	0	0	0	0	0	0	0
Subtotals	H	2	0	0	0	2	3	2	2	0	7	0	0	5	3	8
	S	2	2	2	2	8	8	8	1	2	19	0	0	0	0	0
Non-basidiomycetes																
Alternaria tenuis	H	10	3	3	0	16	8	9	3	1	21	0	0	0	0	0
	S	3	0	0	0	3	15	14	0	1	30	0	0	0	0	0
Epicoccum nigrum	H	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
	S	1	0	2	0	3	2	0	0	0	2	0	0	0	0	0
Ceratocystis piceae	H	33	34	25	14	106	12	22	24	17	75	4	7	20	9	40
	S	37	36	29	13	115	19	31	34	18	102	35	22	23	16	96
Cytospora kunzei	H	1	0	0	0	1	2	0	0	0	2	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichoderma viride	H	0	0	1	2	3	0	1	4	2	7	0	0	1	0	1
	S	0	0	0	2	2	0	1	3	2	6	0	0	0	0	0
Bacterium "J"	H	6	4	10	14	34	11	3	4	19	37	8	14	15	21	58
	S	10	4	2	1	17	10	2	0	0	12	5	0	0	4	9
Bacteria "1"	H	2	10	15	35	62	18	7	12	23	60	28	27	21	31	107
	S	24	23	25	36	108	16	2	15	32	65	31	38	37	33	139
Penicillium sp.	H	0	0	3	0	3	2	0	6	1	9	0	0	0	0	0
	S	0	0	0	2	2	0	0	5	0	5	0	0	1	0	1
Unknown and miscellaneous	H	7	4	2	0	13	9	0	0	1	10	0	0	0	1	1
	S	3	1	2	2	8	2	5	1	2	10	1	0	2	0	3
Subtotals	H	59	55	59	65	238	62	42	53	65	222	40	48	57	62	207
	S	78	64	60	56	258	64	55	58	55	232	72	60	63	53	248
Negative	H	2	0	6	1	9	1	4	4	2	11	4	9	2	0	15
	S	0	1	6	2	9	0	1	0	0	1	0	0	0	1	1

NOTE: Basis: 80 isolations from five sections representing each category.
Exposure periods: 1= 29/7/65 to 31/8/65; 2= 17/8/65 to 13/9/65 (15/9/65 L. Park); 3= 31/8/65 to 30/9/65 (19/10/65 L. Park); 4= 13/9/65 to 13/10/65 (19/10/65 L. Park).
H= heartwood infections; S= sapwood infections; T= totals.

In earlier studies, *S. sanguinolentum* was found colonizing heartwood more frequently than sapwood (Etheridge and Morin 1963). A similar situation is evident in the present work, which consistently shows a greater frequency of isolation of this fungus from heartwood than from sapwood. Colonization by *S. sanguinolentum* also seems to be more successful in injuries of living trees, as seen by the higher frequencies of infection in the decapitated trees than in the excised stem sections, under comparable conditions of exposure. An attempt was made to see whether a similar unfavorable substrate effect could be reproduced in the living trees by deliberately changing the nutrient composition of the wound surface. This was tested by adding appropriate volumes of a 5% malt extract to freshly exposed wound surfaces of both living trees and excised stem sections of balsam fir. It was thought that the nutritional amendment would encourage saprophytes, especially non-decay-producing organisms, which might compete successfully with *S. sanguinolentum* for the modified wound surface. The effect of the treatment on the frequency of isolation of *S. sanguinolentum* and its competitors is given in Table V.

Species showing a marked decrease in the frequency of isolation after the treatment to living trees were *S. sanguinolentum*, *Peniophora*

TABLE V

The effect on the kind and frequency of colonizing microorganisms of 5% malt extract applied to freshly exposed cross-sectional surfaces of living (decapitated trees) and dead (4-in. sections) balsam fir stems for various exposure periods

	Living (30 trees)						Dead (32 stem sections)											
	(26/5/65 to 26/5/66)						(3/5/65 to 3/6/65)						(20/5/65 to 21/6/65)					
	Treat. (15)			Non-treat. (15)			Treat. (8)			Non-treat. (8)			Treat. (8)			Non-treat. (8)		
Organisms	H	S	T	H	S	T	H	S	T	H	S	T	H	S	T	H	S	T
Basidiomycetes																		
Stereum sanguinolentum	1	1	*2*	4	5	*9*	2	0	*2*	2	0	*2*	1	0	*1*	1	0	*1*
Amylostereum chailletii	1	1	*2*	0	0	*0*	0	0	*0*	0	0	*0*	0	0	*0*	0	0	*0*
Stereum purpureum	12	7	*19*	5	4	*9*	0	0	*0*	0	0	*0*	0	0	*0*	0	0	*0*
Peniophora cinerea	1	0	*1*	3	3	*6*	0	0	*0*	0	2	*2*	0	1	*1*	0	0	*0*
Peniophora gigantea	1	4	*5*	2	0	*2*	0	0	*0*	1	0	*1*	1	2	*3*	0	0	*0*
Polyporus adustus	0	6	*6*	1	6	*7*	0	0	*0*	0	0	*0*	0	0	*0*	0	0	*0*
Corticium laeve	16	11	*27*	8	8	*16*	0	1	*1*	1	3	*4*	0	2	*2*	0	1	*1*
Corticium sp.[1]	0	0	*0*	0	0	*0*	1	0	*1*	0	0	*0*	0	0	*0*	0	0	*0*
Unknown and miscellaneous	2	3	*5*	0	1	*1*	1	1	*2*	0	2	*2*	0	5	*5*	0	0	*0*
Subtotals	34	33	*67*	23	27	*50*	4	2	*6*	4	7	*11*	2	10	*12*	1	1	*2*
Non-basidiomycetes																		
Alternaria tenuis	14	11	*25*	8	10	*18*	8	18	*26*	5	13	*18*	4	5	*9*	6	15	*21*
Epicoccum nigrum	2	1	*3*	1	6	*7*	1	2	*3*	3	4	*7*	1	3	*4*	1	6	*7*
Ceratocystis piceae	1	0	*1*	0	0	*0*	5	4	*9*	5	1	*6*	8	0	*8*	13	8	*21*
Ceratocystis sp.	0	0	*0*	0	0	*0*	0	1	*1*	0	0	*0*	1	0	*1*	1	1	*2*
Cytospora kunzei	3	4	*7*	9	7	*16*	14	9	*23*	7	4	11	15	15	*30*	1	1	*2*
Cytospora sp.	0	0	*0*	0	0	*0*	20	13	*33*	8	5	*13*	1	0	*1*	0	0	*0*
Trichoderma viride	0	0	*0*	0	0	*0*	0	0	*0*	0	0	*0*	1	0	*1*	0	0	*0*
Bacterium "J"	1	3	*4*	5	1	*6*	4	0	*4*	4	2	*6*	7	0	*7*	17	2	*19*
Bacteria "1"	13	8	*21*	9	4	*13*	11	24	*35*	29	26	*55*	34	4	*38*	49	6	*55*
Penicillium sp.	0	0	*0*	0	0	*0*	7	8	*15*	0	0	*0*	3	2	*5*	1	4	*5*
Thyronectria sp.	2	0	*2*	8	0	*8*	2	1	*3*	1	3	*4*	10	3	*13*	9	1	*10*
Unknown and miscellaneous	12	8	*20*	8	9	*17*	8	13	*21*	4	12	*16*	11	5	*16*	11	15	*26*
Subtotals	48	35	*83*	48	37	*85*	80	93	*173*	66	70	*136*	96	37	*133*	109	59	*168*
Negative	1	3	*4*	2	5	*7*	10	12	*22*	9	7	*16*	5	25	*30*	5	30	*35*

NOTE: Basis: 16 isolation attempts (eight heartwood, eight sapwood) from each stem sample. H= heartwood infections; S= sapwood infections; T= total.

cinerea, *Epicoccum nigrum* Lk., *Cytospora kunzei*, and *Thyronectria* sp., while those showing a marked increase were *Stereum purpureum*, *Peniophora gigantea* (Fr.) Masse, *Corticium laeve*, *Alternaria tenuis*, and bacteria "1". Generally, the treatment adversely affected colonization by species showing some degree of substrate specificity, namely, *Stereum sanguinolentum*, *Cytospora kunzei*, and *Thyronectria* sp., but unaccountably the treatment favored the latter two fungi on excised stem sections. *Stereum sanguinolentum* and *Peniophora cinerea* showed the greatest percentage of change in the frequency of isolation (about 80%) in response to the treatment whereas, from the standpoint of trees infected, the incidence dropped from 46% to 13% for *Stereum sanguinolentum* and from 40% to 7% for *Peniophora cinerea* after treatment. *Stereum purpureum* and *Corticium laeve* of the decay fungi, and *Alternaria tenuis* of the non-decay fungi, all showed a substantial increase in frequency of occurrence in the heartwood after the treatment, and this was reflected by a corresponding reduction in the frequency of *S. sanguinolentum* infections.

From the standpoint of influencing infection by *S. sanguinolentum*, of "treated" living trees, *S. purpureum* was perhaps the most important fungal competitor. It was absent in all six of the untreated, but infected trees, and present in half of those treated, but uninfected by this pathogen. *Corticium laeve* seemed to be relatively unimportant in infection of the living trees by *Stereum sanguinolentum* as a result of the treatment. *Ceratocystis piceae*, which is one of the principal fungal competitors of *S. sanguinolentum* in excised stem sections, was conspicuously absent in both the treated and non-treated, decapitated, living trees.

It is noteworthy that the addition of malt extract to the living trees had the same unfavorable effect on the frequency of occurrence of *S. sanguinolentum* as that resulting from excision. The treatment had no apparent effect on the already limited capacity of *S. sanguinolentum* to colonize the excised material.

Height of Wound from Ground

The effect of different heights on colonization of balsam fir by *S. sanguinolentum* was investigated by exposing freshly cut stem sections on small platforms installed at 5-ft intervals along the stem of a large balsam fir tree in the study area. The frequency and kind of colonizing microorganisms was determined as in the previous experiments. The periods of exposure and the results of this experiment are presented in Table VI. The species most affected by increasing the elevation of the wound surface was *Peniophora cinerea*, which showed remarkably uniform increases in frequency, and bacteria "1", which was adversely affected by increasing heights.

Generally, infections by decay fungi, including *S. sanguinolentum* which was isolated three times from traps installed at 12 and 20 ft, tended to be favored by higher elevations, while non-decay-producing species were unfavorably affected. For example, traps exposed at ground level produced the greatest number of isolates of imperfect fungi and bacteria, and the least number of basidiomycetous fungi. *Alternaria tenuis*, *Ceratocystis piceae*, and bacterium "J", suspected competitors of *S. sanguinolentum* at higher temperatures, did not appear to be seriously affected by the different elevations.

Forest Stand and Composition

The frequencies and patterns of occurrence of species colonizing wound surfaces of balsam fir have been described heretofore for a single stand in the principal study area at Duchesnay. Colonization of the fresh wound surface was studied also in balsam fir stands at Lac Jaune (near the main area), as well as in Laurentide Park, to allow for comparison of different stand compositions and climatic areas. The results of these studies, for comparable exposure periods during August, September, and October, 1965, are given in Table IV. The major difference between the Duchesnay and Lac Jaune stands is age and the percentage of balsam fir, both of which are greater in Duchesnay. The Laurentide Park stand differed from the other two, being younger, and having a greater percentage of balsam fir, in the apparent absence of slash and of sporophores of *S. sanguinolentum*, and in much lower prevailing temperatures, because of its higher elevation.

The species showing the greatest variation in occurrence over the three areas were *S. sanguinolentum*, which despite the apparent absence

TABLE VI

Kind and frequency of microorganisms isolated from freshly cut balsam fir stem sections exposed periodically at different heights from the ground in a balsam fir stand at Duchesnay, Quebec, during July, August, and September 1965

Organisms		Feet from ground: 0	4	8	12	16	20	24	Totals
		No. isolations							
Basidiomycetes									
Stereum sanguinolentum	H	0	0	0	0	0	1	0	1
	S	0	0	0	1	0	1	0	2
	T	0	0	0	1	0	2	0	3
Peniophora cinerea	H	0	2	1	6	4	4	2	19
	S	3	3	8	9	19	23	13	78
	T	3	5	9	15	23	27	15	97
Other basidiomycetes	H	0	3	0	0	1	0	1	5
	S	1	3	6	2	6	3	6	27
	T	1	6	6	2	7	3	7	32
Subtotals	H	0	5	1	6	5	5	3	25
	S	4	6	14	12	25	27	19	107
	T	4	11	15	18	30	32	22	132
Non-basidiomycetes									
Alternaria tenuis	H	4	7	10	11	7	25	14	78
	S	8	9	5	11	9	8	12	62
	T	12	16	15	22	16	33	26	140
Ceratocystis piceae	H	87	45	81	64	68	55	68	468
	S	116	100	90	113	98	105	113	735
	T	203	145	171	177	166	160	181	1203
Bacterium "J"	H	46	83	61	38	50	42	58	378
	S	7	3	3	9	25	27	18	92
	T	53	86	64	47	75	69	76	470
Bacteria "1"	H	90	90	94	84	73	53	68	552
	S	117	113	105	101	72	74	89	671
	T	207	203	199	185	145	127	157	1223
Other non-basidiomycetes	H	27	19	15	12	29	28	23	156
	S	29	20	17	12	21	44	28	178
	T	56	39	32	24	50	72	51	334
Subtotals	H	254	244	262	209	229	203	231	1632
	S	277	247	222	247	227	258	260	1738
	T	531	491	484	456	456	461	491	3370
Grand totals	H	254	249	263	215	234	208	234	1657
	S	281	253	236	259	252	285	279	1845
	T	535	502	499	474	486	493	543	3502

NOTE: Exposure periods: 13/7/65 to 12/8/65; 12/8/65 to 30/8/65; 30/8/65 to 16/9/65; 16/9/65 to 30/9/65.
Basis: each figure represents the total number of isolates of a particular microorganism obtained from eight isolations attempted from each of five stem sections (40) over four exposure periods (160).
H= heartwood infections; S= sapwood infections; T= totals.

of sporophores was favored by the lower temperatures of the Laurentide Park stand, *Stereum purpureum* and *Peniophora cinerea*, which were most abundant in the Lac Jaune stand, and *Alternaria tenuis*, *Epicoccum nigrum*, *Cytospora kunzei*, and *Trichoderma viride* Pers. ex Fr., all of which were conspicuous by their absence in the Laurentide Park stand. All the species of bacteria were favored by the cooler climate in Laurentide Park. The three suspected fungal competitors of *S. sanguinolentum*; namely, *Peniophora cinerea*, *Alternaria tenuis*, and *Ceratocystis piceae*, which showed reduced frequencies during exposure periods characterized by low temperatures in the main experiment, were either absent or occurred in substantially reduced frequencies in the cooler Park stand.

Discussion

Although suitable conditions for the production of viable basidiospores of *S. sanguinolentum* occurred in the study area from mid-April to mid-November, optimal conditions for release and germination occurred only during extended periods of high relative humidity, usually associated with several days rain and mean daily temperatures of between 45 and 75 F. The optimal range of temperature for successful colonization of the fresh wound surface of balsam fir by *S. sanguinolentum* was between 45 and 55 F, suggesting that factors other than inoculum intensity were decisive in the infection process.

It appears that one of the most important factors limiting infection by *S. sanguinolentum* to ambient temperatures below 55 F is the increased rate at which wound surfaces are colonized by competing microorganisms above this critical temperature. Fresh surfaces of stem sections exposed on days characterized by temperature of 55 F or greater, were almost exclusively colonized by such fungi as *Peniophora cinerea*, *Alternaria tenuis*, *Ceratocystis piceae*, and a yellow-pigmented bacterium (bacterium "J"), species which were absent, or occurred infrequently, at lower temperatures. Most of the successful competitors had growth optima on malt agar in the order of 5 degrees higher than that of *Stereum sanguinolentum*. The single exception, *Ceratocystis piceae*, had similar growth-temperature requirements to those of *S. sanguinolentum* despite the fact that it was the main competitor during warm, dry weather. However, one of the principal means of dissemination of spores in *Ceratocystis* is by insects, particularly bark beetles, which are very sensitive to the influence of temperature and often reach their maximum activity at temperatures in excess of 60 F during sunny, dry periods (Chararas 1962).

Ceratocystis piceae has never been reported to be species specific, but it has been reported associated with several bark beetle genera and certain other insects in Europe (Mathiesen-Kaarik 1960), and there is little doubt from the rapidity at which it colonized traps that associations with insects in the study area have played a major part in its dispersal. Some airborne dissemination of *C. piceae* probably occurred since the fungus was recovered occasionally from traps exposed during extended periods of wet, cool weather when insect activity would be minimal but, because of their rarity, such infections apparently had little competitive effect on *Stereum sanguinolentum*.

In contrast to *Ceratocystis piceae*, colonization of the traps by two air-dispersed species, *Peniophora cinerea* and *Alternaria tenuis*, was favored by ambient temperatures which corresponded to their requirements for mycelial growth on agar. Thus it was not until relatively late in the season, after mean daily temperatures had reached 60 F, that the competitive effect of these two species became significant for *S. sanguinolentum*.

Although temperature appeared to be the decisive factor determining dominance between *S. sanguinolentum* and its competitors, rain had an important bearing on the establishment of this fungus, either directly by providing adequate inoculum and suitable conditions for infection, or indirectly through the selection of competitors for colonization of the wound surface. Although temperatures were often low enough to suppress competitors, infection of the fresh wound surface by *S. sanguinolentum* rarely occurred unless rain had fallen on the day of the injury or on the days immediately after. Rain also appeared to be the decisive factor in determining the competitive dominance of the air-spread, slash-decay fungus, *Peniophora cinerea*, over the insect-disseminated, non-decay fungus, *Ceratocystis piceae*, after only

1 week of exposure of the traps during the relatively warm, wet spell of 19 July to 9 August 1965 (see Fig. 4). Although *P. cinerea* was rapidly replaced in the traps by *C. piceae* after exposure for a second week (Fig. 5), the former fungus was highly effective in preventing the establishment of *S. sanguinolentum* during the critical, initial period of exposure.

The exact nature of the interrelationships between *S. sanguinolentum* and its competitors on the wound surface was not specifically studied. However, several observations suggest that the dominance of the successful invader depends upon its ability to become established on the substrate more rapidly as the result of more favorable and possibly more selective conditions which exist there, and not upon antibiotic activity. It was shown that, whereas basidiospores of *S. sanguinolentum* germinated on the wound surface equally well at 50 and 70 F, germination of conidia of *C. piceae* was markedly arrested at the lower temperature for the initial 24 hours.

Inoculations with these two fungi showed also that despite their close similarity in growth–temperature relations on malt agar, successful infection by *S. sanguinolentum* was obtained on the natural substrate at 50, but not at 70 degrees, whereas infections by *C. piceae* were almost twice as frequent at the higher as at the lower temperature. There was no evidence of antagonism between these two species when grown together on artificial or natural substrates, but the effect of temperature on the interactions of these fungi was not studied and, in the light of recent findings of Henningsson (1967) in Sweden, it is quite possible that different results might be obtained at different temperatures. Low temperatures which inhibit or retard mycelial growth are also very likely to inhibit or retard the production of antibiotics, as has been shown by Michno-Zatorska (1960) for highly antagonistic *Trichoderma viride*.

Freshly exposed heartwood of balsam fir has been shown to act selectively for *S. sanguinolentum* (Etheridge 1962), while certain other substrates are known to affect the optimum temperatures for growth of wood-destroying fungi (Wolpert 1924). One could, therefore, speculate that specific chemical properties of the wound surface may modify the growth–temperature relations of the early invaders to give *S. sanguinolentum* a distinct advantage at lower temperatures.

Physical and chemical differences which occur naturally, or are induced in wood of a given species, may have an important effect on the establishment of wood-inhabiting fungi and this could cause variations in infection levels not directly related to the external environment. Thus, in Alberta, a very low wood moisture content in subalpine spruce on dry sites, in contrast to that on wet sites, was considered to be the major factor responsible for the lower incidence of decay caused by *S. sanguinolentum* and other fungi (Etheridge 1958). Wood moisture content is extremely variable in balsam fir because of the prevalence of wet wood in this species, but observations in Quebec suggest that this factor could have little significance in the establishment of the pathogen in living trees. For example, the moisture content of stem sections successfully infected by *S. sanguinolentum* during the period 23 August to 7 September, 1965, ranged from 78% to 148%, which was about the same range (69% to 130%) recorded for living trees on a variety of sites in the study area (Etheridge and Morin 1962).

The effect on *S. sanguinolentum* of possible differences in the specific gravity of the wood of balsam fir was not investigated. However, on the basis of earlier findings in Quebec (Etheridge and Morin 1962), variations in wood density are not great in this species and occur well within the range of variation in wood density that was found to have a negligible effect on decay in subalpine spruce (Etheridge 1958).

There was no evidence of any connection between substrate suitability and the susceptibility of trees to infection, except for two instances (see Fig. 9). Trees which were injured on 10 June and 25 June 1963, and remained uninfected, were also shown to be most strongly inhibitory against *S. sanguinolentum*. However, both of these injury dates occurred during rainless periods and it was impossible to separate the effects of adverse external conditions for infection and adverse substrate conditions. On the other hand, the markedly greater success of *S. sanguinolentum* on heartwood (Figs. 4 and 5) represents a more consistent observation and suggests that this pathogen benefits from the

exclusion of such fungi as *Ceratocystis piceae*, *Penicillium* spp., and *Peniophora cinerea* by host resistance factors associated with the heartwood in much the same way as has been postulated by Garrett (1960) for *Armillaria mellea* (Vahl. ex Fr.) Kummer. This is supported by the results of earlier studies (Hubbes and Etheridge 1965) which have shown that *S. sanguinolentum* has a greater tolerance for heartwood extractives of recently cut balsam fir than at least two of its commonly occurring competitors, namely *Alternaria tenuis* and *Epicoccum nigrum*.

Several observations point to a rapid breakdown in host resistance and a corresponding increase in the activity of saprophytic competitors of *S. sanguinolentum* when environmental conditions are modified by excision of the substrate from the living host. Thus, it appears reasonably certain that competition by such non-decay microorganisms as *Ceratocystis piceae*, *Penicillium* spp., and bacteria "1", which are favored by the modified heartwood of excised stem sections, is the major reason for the virtual exclusion of *S. sanguinolentum* and other wood-destroying fungi from this substrate.

Similarly, amendment to fresh wound surfaces by a 5% malt extract solution had the effect of increasing the occurrence of such colonizing species as *Stereum purpureum*, *Corticium laeve*, *Peniophora gigantea*, *Alternaria tenuis*, and bacteria "1" to a degree which appreciably reduced infection by the more specialized decay fungi, including *S. sanguinolentum* and *Amylostereum chailletii*. It is significant, from the standpoint of possible application of these findings to the formulation of practical biological control methods against *S. sanguinolentum*, that none of the successful basidiomycetous competitors on the malt-treated wound surfaces have been found associated with advanced decays in living balsam fir; but whether the decay status of these species will remain unchanged by such treatments has not been determined.

It is also possible that some or all of the colonizing species which occur in the wood at an early stage may later be replaced by more aggressive wood-destroying fungi, including *S. sanguinolentum*. The recent observations by Henningsson (1967), which indicate that *Corticium laeve* and *Stereum purpureum* are among the first decay fungi to invade unpeeled birch pulpwood, and that these fungi are later replaced by more pronounced wood-destroyers, support this possibility.

Differences associated with characteristics of the stand and the macroclimate also have an appreciable effect on the distribution of *S. sanguinolentum* and other components of the wound flora of balsam fir. This is evident from the marked differences in occurrence of species colonizing wound surfaces in the three balsam fir stands studied (see Table IV). Thus the major distinguishing stand feature associated with an increase in the occurrence of *S. sanguinolentum* was the cooler climate of the Laurentide Park stand, which is considered to be directly responsible for the reduced frequencies in this stand of *Peniophora cinerea*, *Alternaria tenuis*, and *Ceratocystis piceae*, the three principal competitors of the pathogen in warm situations. It is also noteworthy that the climate of the Laurentide Park stand was relatively unfavorable for the occurrence of *Trichoderma viride*, a fungus which is known to be inhibited by temperatures below 50 F (Michno-Zatorska 1960).

The observations reported here suggest a definition of the range of conditions tolerated by *Stereum sanguinolentum* during the process of infection of balsam fir. The relatively narrow range of temperature requirements and dependence upon rain and apparently freshly produced injuries for the establishment of this fungus in living trees would seem to limit the risk of infection to relatively few periods during the growing season, when conditions are favorable. However, while the susceptibility of summer-produced injuries falls off rapidly for *S. sanguinolentum*, under certain conditions irreversibly, because of intense saprophytic competition for the wound surface, winter-produced injuries, which probably account for most infection courts, are extremely susceptible to this fungus when thawing occurs early in April. It is undoubtedly the superior competitive ability of *S. sanguinolentum* at low temperatures, and the capacity of this fungus to penetrate wood rapidly over a fairly wide range of temperatures, that ensures its continued dominance of the heartwood flora and makes it the most destructive decay pathogen of balsam fir.

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