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Artificial inoculation with *Ips typographus*-associated blue-stain fungi can kill healthy Norway spruce trees

Kunstig inokulering med blåvedsopper som følger granbarkbillen kan drepe friske grantrær

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Ås 1983

Abstract

HORNTVEDT, R., CHRISTIANSEN, E., SOLHEIM, H. & WANG, S. 1983. Artificial inoculation with *Ips typographus*-associated blue-stain fungi can kill healthy Norway spruce trees. (Kunstig inokulering med blåvedsopper som følger granbarkbillen kan drepe friske grantrær.) Medd. Nor. inst. skogforsk. *38*(4): 1—20.

An infection experiment was made to test the pathogenicity of two *Ceratocystis* species on Norway spruce trees. Water stress in inoculated and control trees was measured by means of xylem water potential, stomatal aperture and transpiration stream velocity. Most trees inoculated with *C. polonica*, alone or in combination with *C. penicillata*, became water-stressed. Ten weeks after inoculation almost all sapwood of water-stressed trees was blue-stained at the level of inoculation. Trees inoculated with *C. penicillata* alone did not become water-stressed, and their sapwood was not blue-stained. The results indicate that *C. polonica* is a highly qualified accomplice to *Ips typographus* in its killing of spruce trees.

Utdrag

HORNTVEDT, R., CHRISTIANSEN, E., SOLHEIM, H. & WANG, S. 1983. Artificial inoculation with *Ips typographus*-associated blue-stain fungi can kill healthy Norway spruce trees. (Kunstig inokulering med blåvedsopper som følger granbarkbillen kan drepe friske grantrær.) Medd. Nor. inst. skogforsk. *38*(4): 1—20.

Et infeksjonsforsøk ble utført for å teste patogeniteten av to *Ceratocystis*-arter på grantrær. Tørkestress i inokulerte trær og kontrolltrær ble målt ved hjelp av vannpotensial i kvister, spalteåpningsvidde og transpirasjonsstrømshastighet. De fleste trær inokulert med *C. polonica*, alene eller sammen med *C. penicillata*, viste tørkestress. Ti uker etter inokulering var nesten hele yteveden hos tørkestressete trær blåfarget i inokuleringsnivå. Trær som var inokulert med *C. penicillata* alene hadde ikke blåved og viste ikke tegn til tørkestress. Resultatene indikerer at *C. polonica* er en meget kvalifisert medhjelper til *Ips typographus* når den dreper grantrær.

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Preface

This work is a part of the project «Resistance of conifers to bark beetles, drought and fungi» financed mainly by The Agricultural Research Council of Norway (NLVF). The participation of H. Solheim and S. Wang was made possible by grants from NLVF and NORAD, respectively.

H. Solheim is responsible for the isolation and identification of the fungi used in this work. S. Wang is responsible for the study of transpiration stream velocity by means of ^{82}Br. E. Christiansen and R. Horntvedt are jointly responsible for the rest of the field work, and for the preparation of the manuscript.

Karin Inderberg, Liisa Lassen, Thomas Midttun and Olaug Olsen gave skilful technical assistance. Alan Berryman of Washington State University, Pullman, and Finn Roll-Hansen of the Norwegian Forest Research Institute have critically read the manuscript and given many valuable suggestions. Richard Worrell has made linguistic corrections.

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I. Introduction

Many bark beetles are known to be closely associated with blue-stain fungi. In this mutualistic relationship, the fungus profits from being spread to new hosts as beetles attack trees. The advantage for the beetles may be of various kinds, one role of the fungus may be to render the phloem suitable as a habitat or as food for the beetle progeny (LEACH 1940; GRAHAM 1967).

The pathogenicity of beetle-transmitted fungi to coniferous trees has been a question of much speculation and some experimentation. CRAIGHEAD (1928) suggested that the fungi play an important role in the rapid death of *Dendroctonus*-infested trees. The mere girdling effect of the insects would not cause the death of the trees within a few weeks, because mechanically girdled trees may live on for months or years. As pointed out by Craighead, the fungus seems to kill the trees by interrupting the ascending sap stream. In pines, the tree-killing role of blue-stain fungi has been established experimentally. Several *Ceratocystic* species have been inoculated, some of them producing a wilt which eventually lead to the death of the tree (NELSON & BEAL 1929; NELSON 1934; CAIRD 1935; BRAMBLE & HOLST 1940; MATHRE 1964a, b; BASHAM 1970; HIMELICK 1982). On the other hand, HETRICK (1949) claimed to have observed successful attack and brood development of *Dendroctonus frontalis* Zimm., and also death of the trees, without any blue-stain.

Among the Eurasian scolytids, the spruce bark beetle *Ips typographus* L. ranks as the most active tree-killing species. During outbreaks, millions of Norway spruce (*Picea abies* (L.) Karst.) may be killed. Death may occur within a month of the beetle attack. Several *Ceratocystis* species, as well as other fungi, have been isolated from the blue-stained sapwood of dead trees (LAGERBERG, LUNDBERG & MELIN 1928; GROSMANN 1931; SIEMASZKO 1939; RENNERFELT 1950; MATHIESEN-KÄÄRIK 1953; KÄÄRIK 1975; SOLHEIM in prep.). An early attempt to inoculate spruce trees with *Ceratocystis* spp. failed (MÜNCH 1907—1908). The air content of the sapwood was thought to be crucial for the establishment of the fungi.

To our knowledge, no other attempt has been made to mass-infect spruce trees with beetle-associated blue stain fungi. The present study was designed to show whether these fungi could infect and kill spruce trees after inoculation into the cambial zone, without the participation of bark beetles. Two fungus species were chosen, *Ceratocystis penicillata* (Grosm.) C. Moreau and *C. polonica* (Siem.) C. Moreau, both frequently isolated near the advancing front of blue-stain in beetle-attacked trees (SOLHEIM in prep.). Because the fungi are thought to inhibit the water transport, we also measured water stress in the trees.

II. Material and methods

The study area was in Ås, 30 km south of Oslo, at an altitude of 100 m (UTM grid reference 32VPM002177). Most of the experimental trees grew in a sparsely stocked stand of Norway spruce intermingled with a few Scots pines (*Pinus sylvestris* L.). The soil was a well-drained moraine. Two of the trees (Nos. 21, 22) grew in a nearby dense plantation of Norway spruce on a somewhat moister and more fertile soil.

The trees and the treatments they received are described in Table 1. The trees appeared to be healthy, all having green foliage and showing no significant foliage depletion. They had no stem wounds or signs of earlier beetle attacks. The five semi-mature trees had a higher breast height age than expected, due to a dense core of narrow annual rings. However, these trees had deeper crowns, better growth and a larger proportion of sapwood than the five mature trees.

Air temperature and relative humidity were recorded at 2 m above ground level in a clear-cut area close to the experimental trees. Vapour pressure deficit (VPD) was calculated and used as a criterion correlated to evapotranspiration. June and July of 1981 were wet and cool, August was dry and warm.

Two types of fungus inocula were used: 1) spruce sapwood chips permeated by *C. polonica* mycelium and 2) malt agar with mycelia of *C. polonica* or *C. penicillata*. The wood chips (2×2×50 mm) were made of fresh sapwood, autoclaved at 120° C, and placed in bundles end down on young cultures of *C. polonica* on malt agar for 2 weeks at room temperature. Malt agar and chips without fungi, but otherwise treated as above, served as control inocula.

The wood chips were forced upwards into the cambial area through slits in the bark made with a small chisel. In this way, 1—2 cm of the chips were in contact with the bark under variable air access conditions. When inoculating with agar, a horizontal hole was cut through the bark with a 5 mm cork borer, and the bark plug removed. A small piece of mycelium and/or agar was then inserted and the bark plug put back into the hole.

The inocula were placed 2 cm apart along rings encircling the stem. Four trees were given six rings of fungal inocula, spaced 15—20 cm apart between 0.8 and 1.65 m above stump height, avoiding branch whorls. Chips were used either in the first and fourth ring from below, or in the third and sixth. Inocula of the two fungi on agar were given in random order to each of the two remaining pairs of rings below, between or above the rings with chips. Four control trees were given similar treatments, but with sterile inocula. Another four trees were inoculated with only one fungus each. These trees were given four rings of inocula on agar, spaced 10—15 cm apart between 0.8 and 1.4 m above stump height. Two trees were left untreated.

Adjacent to three of the mature trees (Nos. 6, 9, 10) and three of the semi-mature (Nos. 2, 3, 4), permanent platforms were erected at 15 and 9 m, respectively, to facilitate sampling of twigs. The other trees had to be climbed and were hence sampled less frequently.

Table 1. Description of the experimental trees and their treatments

Tree No.	Treatment (Inoculum)	Age (yr.)	Height (m)	Crown height (m)	Diam. at 1.3 m (cm)	Radial growth last 5 yr. (mm)	Sapwood area at 1.3 m		Stage of tree development
							(cm^2)	(% of total)	
2	Ceratocystis polonica and C. penicillata	86	16.1	2.6	20.4	9.4	205	79	Semi-mature
17	» »	87	14.8	2.9	22.9	7.1	235	74	»
6	» »	98	21.2	11.3	21.3	1.8	138	46	Mature
16	» »	108	19.0	7.6	24.5	5.1	267	67	»
21	C. polonica	31	16.3	5.2	20.2	9.1	140	50	Pole stage
19	»	120	19.6	8.3	23.6	5.5	192	54	Mature
22	C. penicillata	31	16.1	3.7	19.1	13.3	200	72	Pole stage
20	»	98	22.7	7.2	28.8	6.4	352	62	Mature
3	Sterile inocula	97	18.5	4.6	23.6	10.4	277	78	Semi-mature
18	»	59	15.8	2.9	20.8	12.8	237	88	»
9	»	109	22.5	9.3	28.0	3.0	510	56	Mature
15	»	140	18.5	11.4	22.6	0.8	80	24	»
4	Untreated		17.9	3.0	23.2				Semi-mature
10	»		22.5	7.5	35.7				Mature

The water potential in the tree crowns was measured by the pressure bomb technique (SCHOLANDER et al. 1965). On each occasion two twigs were cut at the second to fourth internode (depending on shoot length) from the mid-region of the crown. The twigs were kept for maximum 10 minutes in closed and shaded polyethylene bags until measurement. Pilot studies had shown that the water potential of twigs stored in this way did not change significantly in the course of 30 minutes.

Stomatal aperture of the needles was estimated by infiltrating them in a solution of methyl violet. Having tested various solvents (SLAVÍK 1974), we found the method recommended by MICHAEL (1969) to be best. Second year shoots were submerged, tip down, for two minutes in a saturated solution of methyl violet in equal amounts of butane-2-ol and diethylether, then quickly rinsed in butane-2-ol, and finally sprayed thoroughly with water. Infiltration was estimated visually on the following scale, slightly modified from MICHAEL (1969):

0 = no infiltration, only wax plugs coloured
1 = infiltration of some guard cells
2 = up to 50 per cent of the guard cells and some larger spots infiltrated
3 = 50—90 per cent of the guard cells infiltrated. Larger spots more frequent; but still isolated
4 = 90—100 per cent of the guard cells infiltrated. Larger spots frequent and partly coalescing
5 = large infiltration spots all over the needle

The transpiration stream velocity in the stems was measured by injecting a gamma ray emittor, ^{82}Br, into the stem and following its movement by means of a scintillation counter (WANG in prep.). At 0.5, 2, and at 9 or 15 m above ground a longitudinally halved polyethylene test tube, 1×8 cm, was glued onto the bark. The tube was filled with water, a horizontal hole, 2 mm wide, was drilled through the tube close to the lower end, and 1.5 cm into the wood. The hole in the tube was then sealed before all the water escaped. 3 ml of $NH_4{}^{82}Br$ solution (4.5 μCi/ml) was added to the tube and the radioactivity measured at 10 cm intervals above the hole every half hour. Additional $NH_4{}^{82}Br$ solution was given when needed. To reduce background radiation, the head of the detector was covered by a lead cap with a narrow slit in the front.

At the end of the experiment, all trees except the untreated controls were felled. Disks 5 cm thick were cut at the inoculation rings and at about 0.2, 0.5, 1.9, 2.2, and at 9 or 15 m above stump height for determination of water content and reisolation. At each level and additional disk, 0.5 cm thick, was cut for delineation and area calculation of sapwood, blue stain and other discolourations. Wood blocks, 2×2 cm, were split from the 5 cm disk along a N-S diameter. The positions of these blocks were also marked on the adjoining 0.5 cm disk. The blocks were weighed fresh, then dried at 105° C to constant weight and reweighed. Water content was calculated as percentage of dry weight.

The trees were inoculated during 15—17 June 1981. Measurement of water potential and stomatal aperture of the trees accessible from platforms

started before inoculation and continued at one-week intervals until harvesting, except for a three-week pause in July. The measurements were done at least twice daily, at noon and either in the evening after sunset or in the morning before sunrise («night values»). The trees which were not accessible from platforms, were measured only once or twice just prior to harvesting. Transpiration stream velocity was measured on the day of harvesting, which took place on 21—29 August.

III. Results

A. Water potential and stomatal aperture

Water potential and stomatal aperture for three of the test trees a day before inoculation are shown in Fig. 1. A rainy night was followed by a bright day with a maximum temperature of 21.6° C and 10.6 h of sunshine. The vapour pressure deficit (VPD) of the air increased rapidly until 1 p.m., then more slowly until 5.30 p.m. The water potential followed the increase in VPD until noon, then levelled off as the stomata gradually closed. Between 6 and 10 p.m. the rapid decrease in VPD caused a parallel fall in the water potential. With a constant low level of VPD during the night and with the stomata partly closed, the trees were able to slowly reduce their water potential until next morning. The stomata movements during the night were unexpected by us, but have been observed in succulents (e.g. EHRLER 1971).

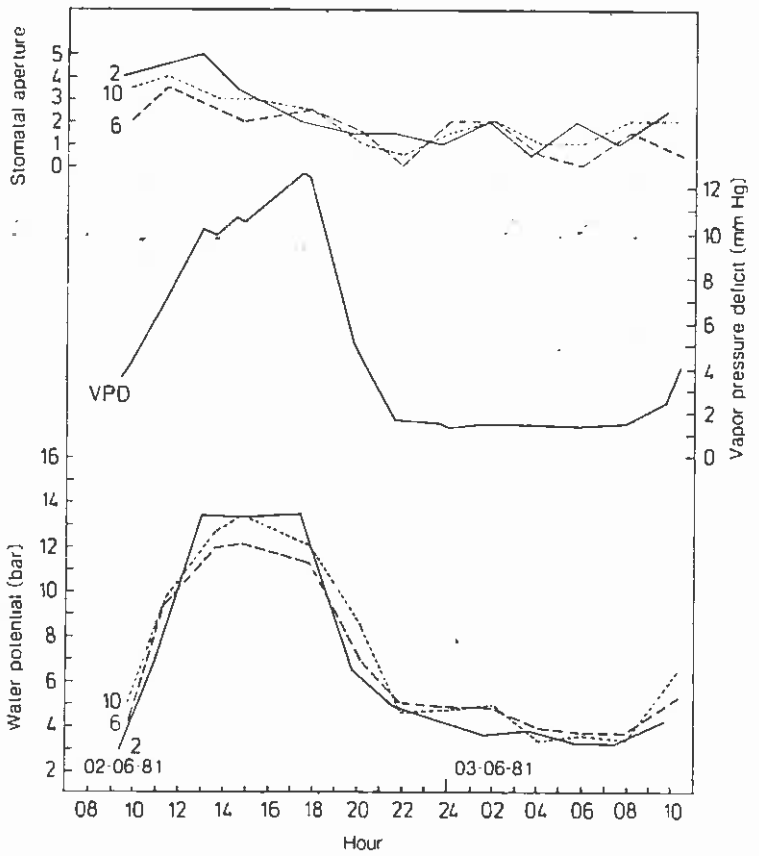

Fig. 1. Water potential and stomatal aperture of three trees before inoculation, together with vapour pressure deficit of the air.

Values for 5—6 August, six weeks after inoculation, are shown in Fig. 2. The weather of 5 August was calm and cloudy before noon, followed by a period of slight breeze and some sunshine from 1 to 4 p.m., then cloudy again. The stomata of the two inoculated trees opened much less than those of the controls. The water potential reached about the same maximum level by noon in all the measured trees, then in the two inoculated trees it decreased very slowly or not at all.

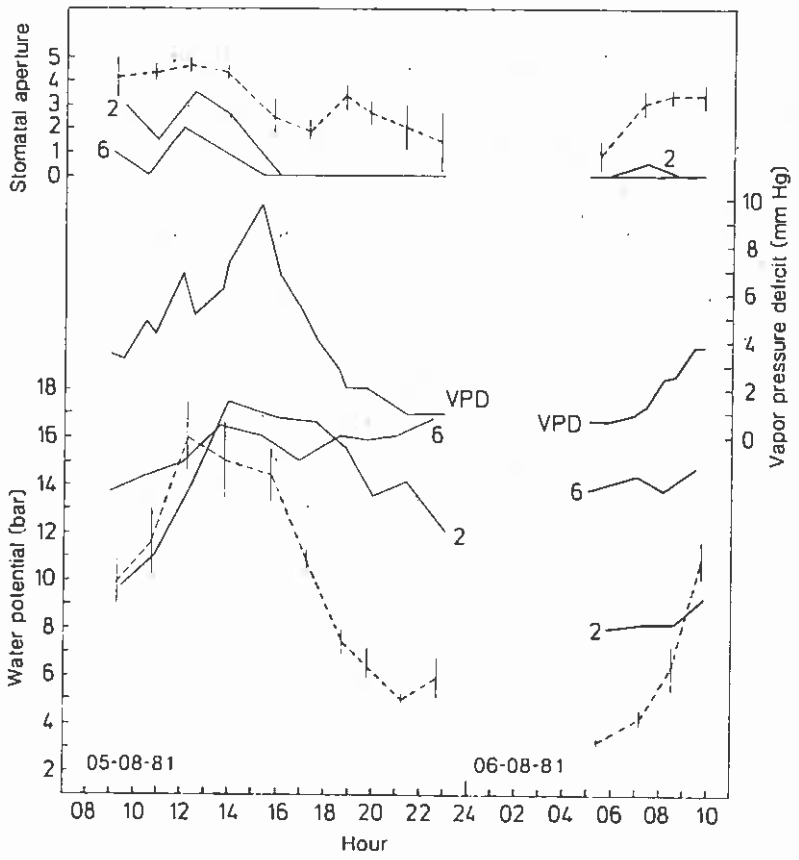

Fig. 2. Water potential and stomatal aperture six weeks after inoculation. Values for two trees inoculated with both fungi (Nos. 2 and 6) and mean values with confidence limits of two control and two untreated trees (stippled lines).

Fig. 3 shows the time development of water potential at night and stomatal aperture at mid-day, for the trees measured most frequently. The water potential of control trees apparently increased during the summer. This may in part reflect increasing water stress during the summer months, especially in August. The water potential of the two inoculated trees remained at the same level as that of the control trees until early July, when at least one tree (No. 6) started to change pattern. The stomatal aperture showed similar changes with time as did the water potential. Tree No. 6 reacted first, then No. 2.

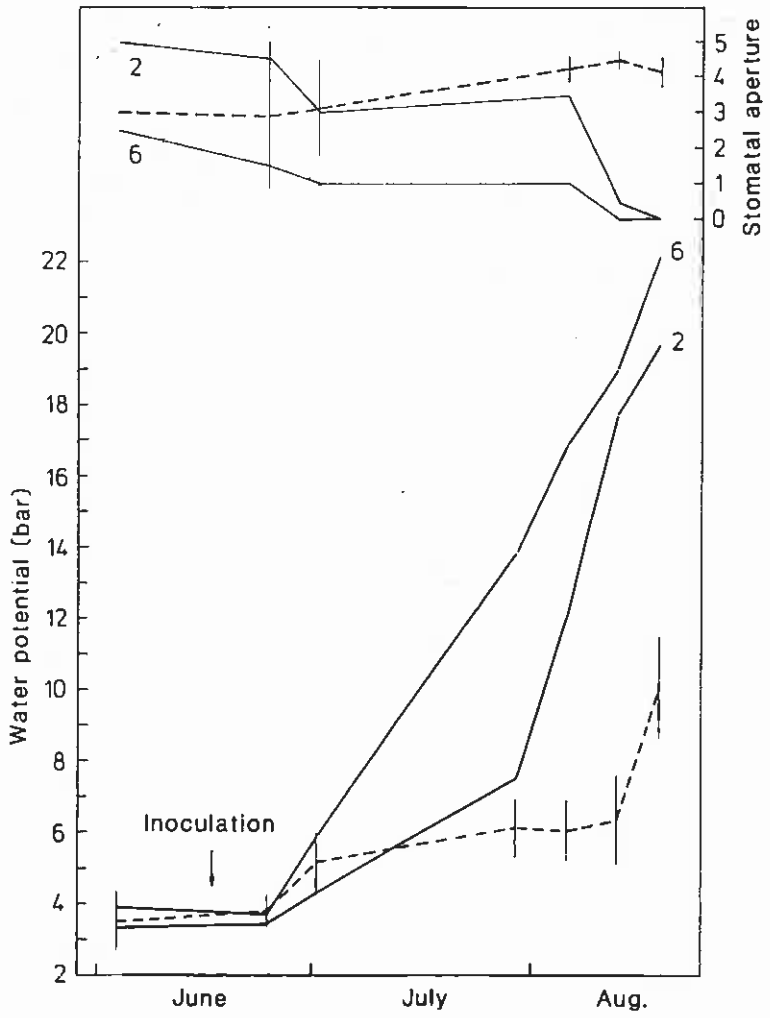

Fig. 3. Water potential at night and stomatal aperture at mid-day during summer 1981. Values for two trees inoculated with both fungi (whole lines) and mean values with confidence limits for control and untreated trees (stippled lines).

Values for all trees by late August are given in Table 2. Neither water potential nor stomatal aperture of trees with sterile inocula deviated significantly from those of untreated trees. The same applies to trees inoculated with *C. penicillata* only. Most of the trees inoculated with either *C. polonica* alone or with both fungi, showed increased water potential by night, and/or reduced stomatal aperture by noon. Only one tree in this group (No. 16) exhibited no signs of water stress. In this tree, fungal penetration into the sapwood, as judged from blue-stain, was much less extensive than in the other trees.

B. Transpiration stream velocity

The ascent of the injected radio-isotope at three heights in six trees is shown in Fig. 4. Often, the ascent was faster during the first hour after injection than later. This initial «leap» (stippled line) is regarded by WANG (In prep.) to be due to a release of the tension in the water columns of the

Table 2. Water potential at night, stomatal aperture at mid-day, blue-stained fraction of sapwood, and maximum vertical distance of blue-stain from the inoculation rings by late August

Tree No.	Treatment	Date	Water potential (bar)	Stomatal aperture	Blue-stained fraction of sapwood (%)	Vertical growth of blue-stain (cm)
2	C.penicillata & C. polonica	18.8.	19.6	0	97	67
17	»	»	12.4	1.0	100	61
6	»	»	22.1	0	86	70
16	»	»	6.9	3.0	37	37
21	C. polonica	27.8.	25.0	0	100	67
19	»	»	21.0	0.5	100	70
22	C. penicillata	»	12.6	3.5	0	0
20	»	»	8.0	3.0	0	0
3	Sterile inocula	18.8.	8.7	4.0	0	0
18	»	»	8.9	4.0	0	0
9	»	»	9.3	3.5	0	0
15	»	»	9.6	4.0	0	0
4	Untreated	18.8.	11.6	4.5		
10	»	»	11.9	4.5		
4	»	27.8.	9.4	3.5		
10	»	»	12.1	4.0		

xylem, and does not reflect the real transpiration stream velocity. In the hours following, the slope of the lines is about the same at all heights for the two untreated trees, as well as the two treated with sterile inocula. For these four trees, a regression model describing the ascent during this period is: (ascent height in cm) = 7.11 + 0.65 (time from injection in min) + 2.90 (injection height in m). This model explains 86 % of the variation in ascent height. Both regression coefficients, but not the constant term, are significantly different from zero. There is no interaction of time and injection height. The average transpiration stream velocity of the measured trees without fungal inocula was thus $0.65 \times 60 = 39$ cm h^{-1} on this particular day.

In the two trees inoculated with both fungi, the transpiration stream had almost stopped at the levels below and just above the inoculation rings. Only at the highest level of one of the trees, a slight transpiration stream could still be recorded.

C. Blue-stain and water content of the wood

The trees inoculated with blue-stain fungi exhibited a much richer resin flow on the bark surface than did those treated with sterile inocula (Fig. 5 A, B). The wounds of the latter were sealed off from the surrounding tissue by

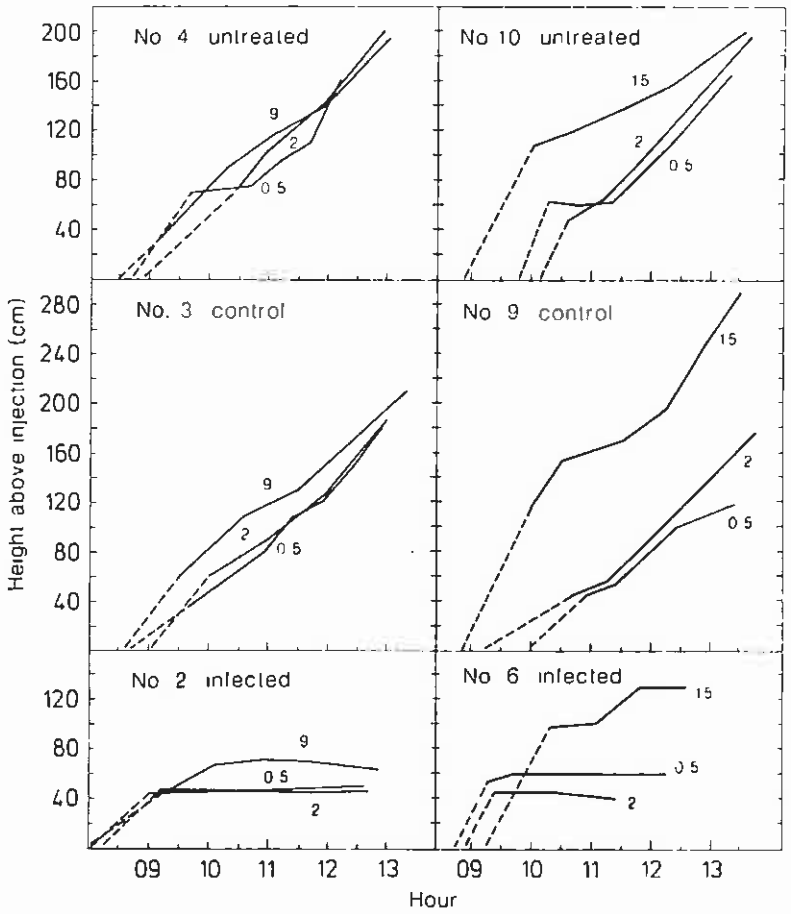

Fig. 4. The ascent of ^{82}Br solution in two infected (inoculated with both fungi), two control and two untreated trees, at the injection levels (m) indicated. Date: 20—21 August. Weather: Calm, partly cloudy, maximum temperature 18—19° C.

a narrow resinous zone (Fig. 5 C), and no visible infection could be detected. Both fungi were able to infect the phloem and produced elliptical areas of brownish, partly resin-infiltrated necroses around the inocula (Fig. 5 D). As judged from the occurence of blue stain, only *C. polonica* was able alone to infect the sapwood. In most cases it had completely penetrated the sapwood at the level of the inoculation rings (Fig. 5 F). *C. penicillata* did not apparently grow into the sapwood of trees where this species alone had been inoculated. Here, wedge-shaped resinous zones could be seen in the sapwood adjacent to the inocula (Fig. 5 E).

In three of four trees inoculated with both fungi, the sapwood inside the inoculation rings was almost completely blue-stained (Table 2). The stained zones originating from the individual inocula had usually joined up, both in the horizontal and vertical direction. Equally large fractions of the sapwood were stained inside *C. penicillata* and *C. polonica* rings, and there was apparently no difference in efficiency between chips and agar as inoculum media. One tree (No. 16) was considerably more resistent than the others to infection (Table 2). Here, an apparently protective response by the resin-infiltrated zones of the sapwood could be seen (Fig. 5 G). This occurred with both fungi.

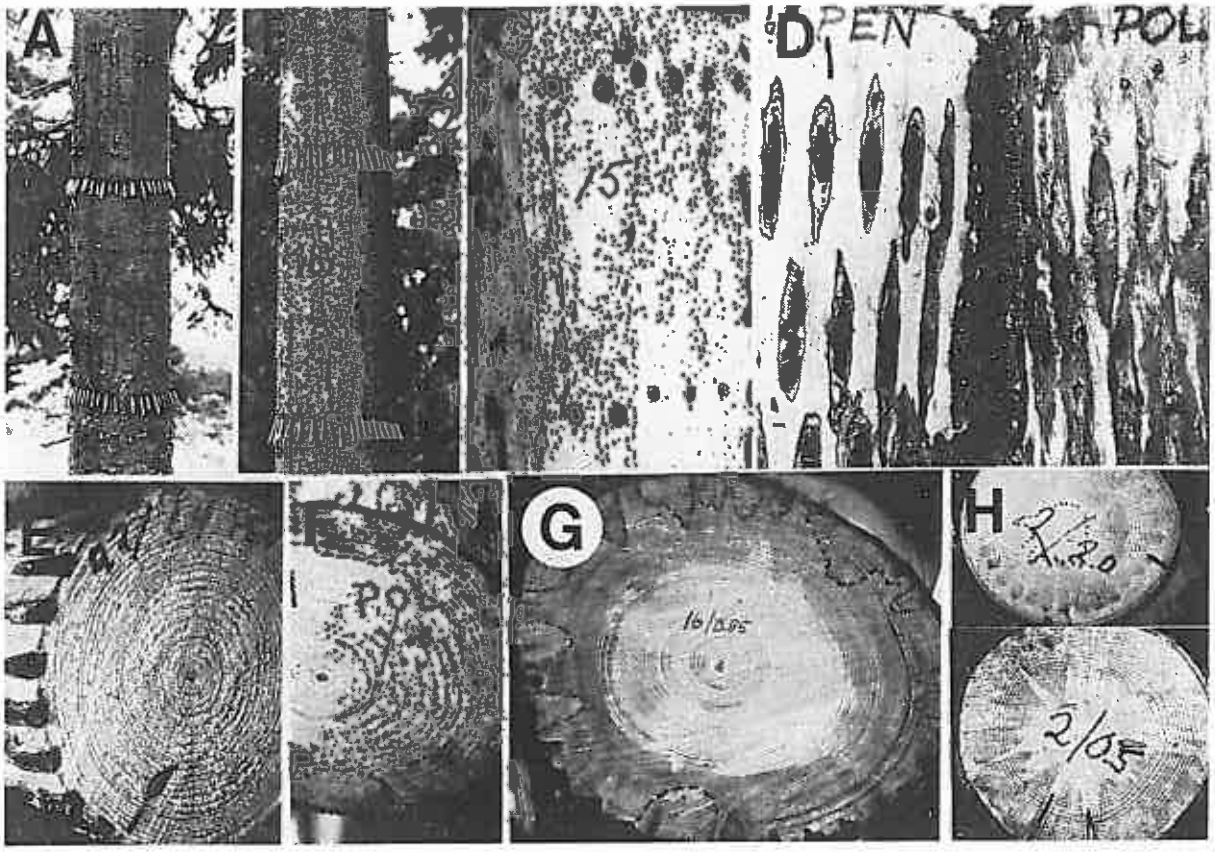

Fig. 5. Reactions of phloem and sapwood to inoculation with blue-stain fungi A. Heavy resin flow on the bark surface of a tree inoculated with both fungi. B. Slight resin flow on a tree with sterile inocula. C. No necroses or discolourations in the phloem surrounding sterile inocula. D. Elliptical necroses, discolourations and resin infiltration of the phloem around inocula with *C. penicillata* (left) and *C. polonica* (right). Secondary death of all phloem between the *C. polonica* zones. E. Resin infiltration, but no penetration of *C. penicillata* into the sapwood. F. Complete penetration of sapwood by *C. polonica*. G. Partial penetration of the sapwood in a tree inoculated with both fungi. Heavy resin infiltration has apparently inhibited fungal growth at some inocula. H. Discs well below and above the inoculation rings. The fungi seem to avoid the outer annual rings.

Below and above the inoculation rings the continuous blue-stain split into individual bodies, wedge-shaped in cross section and gradually tailing off from the outer annual rings (Fig. 5 H). Blue-stain could be detected about 60 cm up and down from the highest and lowest rings, respectively (Table 2). This is equivalent to a growth rate of 25 cm per month in the longitudinal direction. A total penetration of the sapwood in the radial direction during the experimental period is equivalent to a radial growth rate of at least 2 cm per month.

Reisolation from trees inoculated with both fungi indicated that only the two inoculated fungi were present at the advancing zone of blue-stain (Table 3, positions 1 and 2). Here, *C. polonica* was most frequently found. In older parts of blue-stain (positions 3 and 4) both fungi occurred, often intermingled.

A summary of stem wood water contents is given in Table 4. Trees inoculated with *C. penicillata* only, are not included in this analysis. The sapwood of infected trees, including both blue-stained and healthy, had about

Table 3. Results of reisolation from trees inoculated with both fungi. pol = *C. polonica*, pen = *C. penicillata*, p/p = both pen and pol, + = other fungi, 0 = no growth, — = no isolation

Tree No.	Vertical position (m)	Horizontal position[1]			
		1	2	3	4
2	0.95 (pen-ring)	0	pol	pen	p/p
2	1.10 (pol-ring)	0	pol	p/p	p/p
2	1.80 (25 cm above pol-ring)	0	pol	—	—
6	1.05 (pol-ring)	0	pol	pol	p/p
6	1.20 (pen-ring)	0	pen	p/p	p/p
6	1.90 (30 cm above pol-ring)	0	pol	—	—
16	0.50 (35 cm below pen-ring)	pol	pol	—	—
16	1.25 (pol-ring)	pol	pol	pen	+
16	1.40 (pen-ring)	pol	pol	p/p	p/p
17	1.05 (pen-ring)	pen	pen	pen/+	p/p
17	1.20 (pol-ring)	0	pol	pol	pol/+
17	1.90 (25 cm above pol-ring)	pol	pol	—	—

[1] 1 = 0—5 mm ahead of visible blue-stain
2 = just inside visible blue-stain
3 = in the middle of visible blue-stain
4 = near the inoculum

half the water content of control trees. In control trees, the water content of the stem decreased from 150—200 per cent in the outer sapwood to 30—40 per cent in the heartwood. In trees inoculated with both fungi, or with *C. polonica* only, the sapwood water content at the level of the inoculation rings approached that of heartwood. It was also significantly reduced above and below the rings. In Fig. 6 two typical examples are illustrated. In trees inoculated with *C. penicillata* only, the water content of the outer two centimeters of the sapwood, at the levels of inoculation, was intermediate between that of control and infected trees. Elsewhere in these trees the sapwood had normal water content.

Table 4. Water content of stem wood

Wood type	Water content (% of d.w.)		
	25-percentile	Median	75-percentile
Heartwood — all trees	32.7	34.1	37.3
Sapwood — infected trees[1]	37.4	73.3	115.5
Sapwood — control trees[2]	130.9	154.1	170.2

[1] Trees inoculated with both fungi or with *C. polonica* alone
[2] Trees with sterile inocula

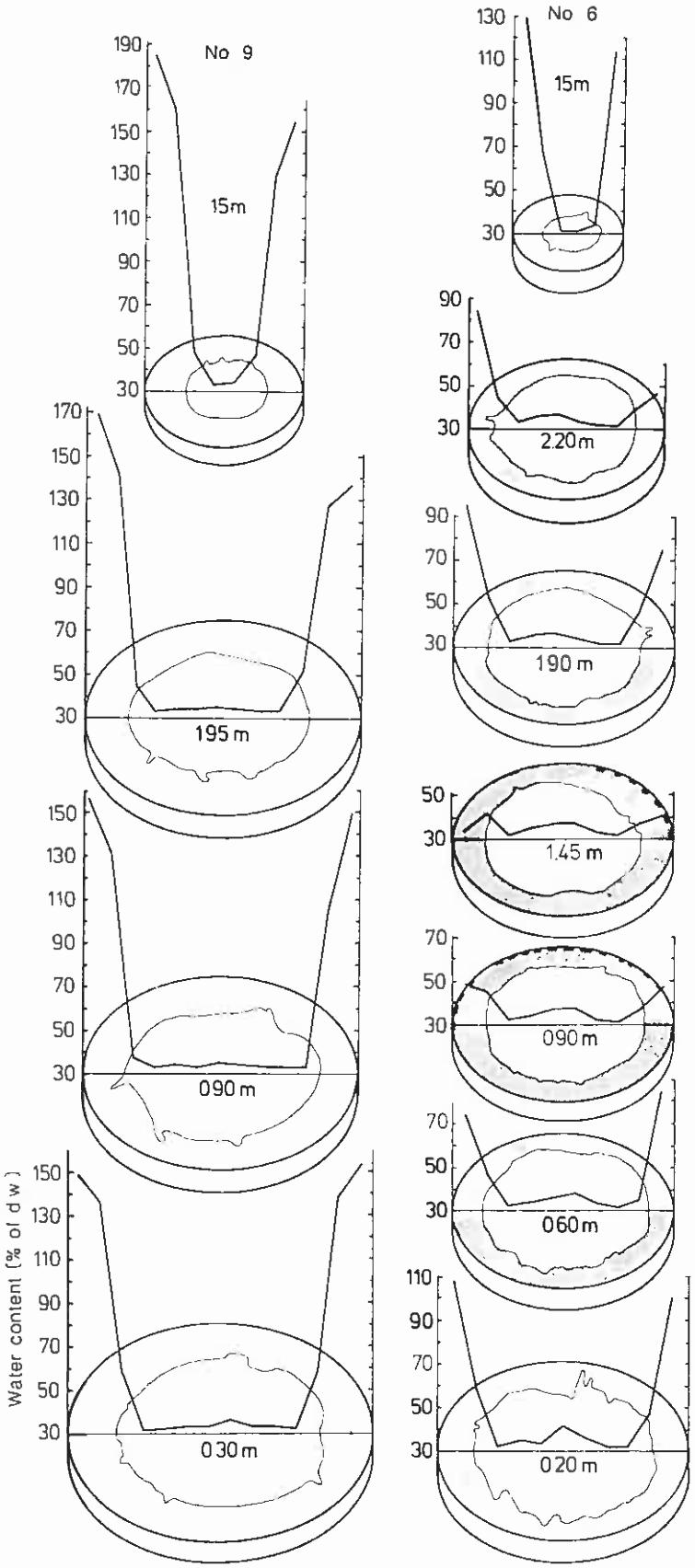

Fig. 6. Water content of stem discs from one tree with sterile inocula (No. 9) and one tree infected with both fungi. The sapwood — heartwood borderline, blue stain (shaded) and resin infiltrations (black) are indicated.

IV. Discussion

The present experiment clearly demonstrates that when adequately inoculated, at least one of the blue-stain fungi normally associated with *Ips typographus* may kill healthy Norway spruce trees. Hence, the tree killing capacity of the beetles may be contingent on the participation of these fungi.

During an attack, the boring activity of bark beetles influences the conditions for both the fungus and the host tree. A single beetle probably carries a smaller dose of inoculum than we used at each point. However, the beetles' gallery construction is an effective way of spreading the spores over a larger area, hence increasing the inoculum potential. Furthermore, the opening up of the bark will cause an initial desiccation and aeration which may facilitate fungal establishment (REID, WHITNEY & WATSON 1967). Recent studies (RAFFA & BERRYMAN in prep.) suggest that trees will survive a higher density of artificial inoculations by the cork borer method than of natural beetle attacks.

The present experiment with a radioactive bromine solution confirms earlier evidence from dye experiments (NELSON 1934; CAIRD 1935; BRAMBLE & HOLST 1940; MATHRE 1964a, b; BASHAM 1970) that blue-stain stops water conduction through the infected sapwood.

Our measurements of water potential and stomatal aperture suggest that a fairly large part of the sapwood cross section must be infected before the water transport is notably inhibited. A similar conclusion was reached by NELSON (1934). It is an open question whether this would be true for a longer period of time or during a prolonged drought.

Instead of applying relatively laborious water status measurements, we might just have observed the discolouration of the foliage of dying trees. In our experience, however, severely water-stressed trees are often invaded by other bark beetles long before their needles become discoloured. *Polygraphus poligraphus* L., in particular, seems capable of detecting such trees. In case of such an attack, it would be difficult to prove that the primary reason for the death of the trees was the artificially inoculated blue-stain fungi.

In trees where the blue-stain had penetrated the whole sapwood, the latter became almost as dry as the heartwood. Similar moisture changes have been observed in beetle-attacked lodgepole pine (REID 1961). The blue-stain fungi grow mainly in the ray parenchyma cells but are also present in the tracheids (BASHAM 1970; WONG & BERRYMAN 1977, among others). The death of infected parenchyma eventually leads to the aspiration of the bordered pits between tracheids, i.e. the tori become deflected to the sides of the pits (NELSON 1934; BASHAM 1970). The aspirated pits are less permeable than normal pits. By the transpiration pull, water is removed from the infected region without sufficient replacement, and the wood gradually becomes dehydrated. Embolism by air and possibly other substances finally causes the irreversible stop of the water conducting capacity.

BASHAM (1970) found that loblolly pine seedlings (2 yr) were more susceptible than saplings (13 yr) to infection with blue-stain fungi. Saplings that were suppressed or of small stem diameter were more susceptible than more vigorous saplings. Although *Ips typographus* can kill both young and old trees, it is by far most harmful to mature or over-mature stands. Our

results do not support a hypothesis that virtually mature trees are more susceptible to infection by blue-stain fungi than are physiologically younger trees. *Ips typographus* mainly kills mature trees, this may probably be due to other factors, such as initial attraction, amounts of resin, or microclimatic conditions.

When attacking a living conifer tree, bark beetles have to cope with more or less abundant amounts of resin, originating from one or two sources (BERRYMAN 1972). In some tree species, such as pines and spruces, primary resin is immediately released from resin channels which are severed by the boring beetles. Later, secondary resinosis may occur in reaction zones surrounding the attack points of the beetles and their associated fungi (REID et al. 1967; BERRYMAN 1969).

Copious amounts of resin is a serious obstacle to normal development of eggs and larvae, and in the bark of trees which resist a beetle attack, resin may continue to be produced for a long period of time. When, however, the blue-stain fungi penetrate the sapwood, kill the living cells, and stop the water transportation the desiccated tree is no longer capable of resinosis.

Of the two fungus species used in our experiment, *Ceratocystis polonica* was the most aggressive, and penetrated the sapwood of the trees. Furthermore, in trees inoculated with both fungus species, *C. polonica* was most frequently found close to the advancing zone of blue-stain in the sapwood. Further experiments are in progress to test the pathogenicity of a larger number of fungi associated with *Ips typographus.*

V. Summary

Two *Ceratocystis* species, frequently isolated from blue-stained sapwood in trees killed by *Ips typographus,* were used in an infection experiment. Eight trees were inoculated in mid-June with *C. penicillata* and *C. polonica,* alone or in combination. Four trees received sterile inocula. Inocula were placed in small holes in the bark, 2 cm apart, in 4—6 rings encircling the stems at 0.8—1.65 m above stump height. Water stress in the trees was measured during the summer until they were harvested in late August.

C. polonica infected both bark and sapwood. *C. penicillata* could infect sapwood together with *C. polonica,* but alone it apparently only infected the bark. Within the experimental period of 70 days, the sapwood inside the inoculation rings could become almost completely blue-stained, equivalent to a radial growth rate of at least 2 cm per month.

Signs of water stress, i.e. high water potential in the crowns at night and low stomatal aperture at daytime, were evident in late July in some trees inoculated with both species, and progressed during August. Transpiration stream velocities by late August were zero in the measured inoculated trees and about 40 cm per hour in control and untreated trees. The water content of extensively blue-stained sapwood approached that of heartwood (30—40 % of d.w.), in contrast to a median water content of 150 % of d.w. in sapwood of control trees.

The results indicate that *C. polonica* is a highly qualified accomplice to *Ips typographus* in its killing of spruce trees.

Kunstig inokulering med blåvedsopper som følger granbarkbillen kan drepe friske grantrær

Granbarkbillen *(Ips typographus)* og de fleste andre barkbiller som angriper levende trær, fører med seg blåvedsopper. Mange av disse hører til slekten *Ceratocystis.* Spiller disse noen rolle i billenes kamp for å ta livet av trærne? Det arbeid som rapporteres her, tok sikte på å undersøke om visse blåvedsopper, når de er inokulert (innpodet) på en liknende måte som den barkbillene benytter, er i stand til å drepe friske grantrær. Dette kunne skje ved at en eller flere blåvedsopper har evne til å vokse inn i yteveden, og derved forårsake at vanntransporten i stammen stopper opp og treet tørker.

To *Ceratocystis*-arter som ofte er isolert fra trær drept av granbarkbillen, ble brukt i et infeksjonsforsøk. Åtte trær ble inokulert i midten av juni med *C. penicillata* og *C. polonica,* alene eller i kombinasjon (tabell 1). Fire trær fikk sterile inokula. Inokula ble plassert i små hull i barken, med 2 cm avstand langs 4—6 ringer rundt stammen, 0,8—1,65 m over stubbehøyde. Trærnes vannstatus ble målt ved flere metoder i løpet av sommeren inntil de ble felt i slutten av august.

C. polonica infiserte både bark og yteved (fig. 5). *C. penicillata* kunne infisere yteved når den var inokulert sammen med *C. polonica,* men alene infiserte den tilsynelatende bare barken. I løpet av forsøksperioden på 70 dager kunne yteveden innenfor inokuleringsringene bli nesten fullstendig gjennomvokst av blåvedsopper (tabell 2). Dette tilsvarer en radial veksthastighet på minst 2 cm pr. måned.

Tegn til tørkestress, dvs. høyt vannsug i kronen om natten og helt eller delvis lukkete spalteåpninger om dagen, var tydelig fra og med slutten av juli hos enkelte trær inokulert med begge sopper (fig. 1, 2 og 3, tabell 2). Transpirasjonsstrømshastigheten i stammen var i slutten av august null i de målte soppinokulerte trærne, mot ca. 40 cm pr. time i trær med sterile inokula og ubehandlete trær (fig. 4). Vanninnholdet i blå yteved var nesten like lavt som i kjerneved (tabell 4, fig. 6).

Resultatene indikerer at blåvedsoppen *C. polonica* er en meget kvalifisert medhjelper til granbarkbillen når den dreper grantrærne. Ved at blåvedsopper gjennomvokser yteveden og treet tørker, mister det evnen til å skille ut kvae, hvilket sannsynligvis er nødvendig for utviklingen av billenes egg og larver.

Literature

BASHAM, H. G. 1970. Wilt of loblolly pine inoculated with blue-stain fungi of the genus *Ceratocystis.* Phytopathology *60*: 750—754.

BERRYMAN, A. A. 1969. Responses of *Abies grandis* to attack by *Scolytus ventralis* (Coleoptera: Scolytidae). Can. Entomol. *101*: 1033—1041.

— 1972. Resistance of conifers to invasion by bark beetle-fungus associations. BioScience *22*: 598—602.

BRAMBLE, W. C. & HOLST, E. C. 1940. Fungi associated with *Dendroctonus frontalis* in killing shortleaf pines and their effect on conduction. Phytopathology *30*: 881—899.

CAIRD, R. W. 1935. Physiology of pines infested with bark beetles. Bot. Gaz. *96*: 709—733.

CRAIGHEAD, F. C. 1928. Interrelation of tree-killing barkbeetles *(Dendroctonus)* and blue stains. J. For. *26*: 886—887.
EHRLER, W. L. 1971. Periodic nocturnal stomatal opening of *Citrus* in a steady environment. Physiologia Pl. *25*: 488—492.
GRAHAM, K. 1967. Fungal-insect mutualism in trees and timber. A. Rev. Ent. *12*: 105—126.
GROSMANN, H. 1931. Beiträge zur Kenntnis der Lebensgemeinschaft zwischen Borkenkäfern und Pilzen. Z. ParasitKde *3*: 56—102.
HETRICK, L. A. 1949. Some overlooked relationships of southern pine beetle. J. econ. Ent. *42*: 466—469.
HIMELICK, E. B. 1982. Pine blue-stain associated with the pine wilt syndrome. J. Arboric. *8*: 212—216.
KÄÄRIK, A. 1975. Succession of microorganisms during wood decay. Pp. 39—51 *in*: LIESE, W. (Ed.). Biological transformation of wood by microorganisms. Proceedings of the Sessions on Wood Products Pathology at the 2nd International Congress of Plant Pathology, September 10—12, 1973, Minneapolis/USA.
LAGERBERG, T., LUNDBERG, G. & MELIN, E. 1928. Biological and practical researches into blueing in pine and spruce. Svenska SkogsvFör. Tidskr. *25*(1927): 145—272, 561—739.
LEACH, J. G. 1940. Insect transmission of plant diseases. McGraw-Hill Book Co., New York.
MATHIESEN-KÄÄRIK, A. 1953. Eine Übersicht über die gewöhnlichsten mit Borkenkäfern assoziierten Bläuepilze in Schweden und einige für Schweden neue Bläuepilze. Meddn St. SkogsforskInst. *43*(4): 1—74.
MATHRE, D. E. 1964a. Survey of *Ceratocystis* spp. associated with bark beetles in California. Contr. Boyce Thompson Inst. *22*: 353—362.
— 1964b. Pathogenicity of *Ceratocystis ips* and *Ceratocystis minor* to *Pinus ponderosa*. Contr. Boyce Thompson Inst. *22*: 363—388.
MICHAEL, G. 1969. Eine Methode zur Bestimmung der Spaltöffnungsweite von Koniferen. Flora *159 A*: 559—561.
MÜNCH, E. 1907—1908. Die Blaufäule des Nadelholzes. Wiss. Z. Forst-u. Landw. *5*: 531—573 (Kap. I—II); *6*: 32—47 (Kap. III), 297—323 (Kap. IV—VII).
NELSON, R. M. 1934. Effect of bluestain fungi on southern pines attacked by bark beetles. Phytopath. Z. *7*: 327—353.
NELSON, R. M. & BEAL, I. A. 1929. Experiments with bluestain fungi in southern pines. Phytopathology *19*: 1101—1106.
RAFFA, K. F. & BERRYMAN, A. A. Influence of fungal inoculation density on the defensive response of lodgepole pine, and its relevance to bark beetle attack thresholds. [In prep.]
REID, R. W. 1961. Moisture changes in lodgepole pine before and after attack by the mountain pine beetle. For. Chron. *37*: 368—403.
REID, R. W., WHITNEY, H. S. & WATSON, J. A. 1967. Reactions of lodgepole pine to attack by *Dendroctonus ponderosae* Hopkins and blue stain fungi. Can. J. Bot. *45*: 1115—1126.
RENNERFELT, E. 1950. Über den Zusammenhang zwischen dem Verblauen des Holzes und den Insekten. Oikos *2*: 120—137.
SCHOLANDER, P. F., HAMMEL, H. T., BRADSTREET, E. D. & HEMMINGSEN, E. A. 1965. Sap pressure in vascular plants. Science *148*: 339—346.
SIEMASZKO, W. 1939. Zespoly grzybów towarzyszacych kornikom polskim. (Fungi associated with bark-beetles in Poland.) Planta pol. *7*(3): 1—54.
SLAVÍK, B. 1974. Methods of studying plant water relations. Ecological Studies 9. Academia, Publishing House of the Czechoslovak Academy of Sciences, Prag. Springer-Verlag, Berlin-Heidelberg-New York.
SOLHEIM, H. Species of Ophiostomataceae isolated from *Picea abies* infested by the bark beetle *Ips typographus*. Nord. J. Bot. [In prep.]
WANG, S. Transpiration stream velocity studied by means of ^{82}Br in *Picea abies* (L.) Karst. attacked by bark beetles and blue stain fungi. Medd. Nor. inst. skogforsk. [In prep.]
WONG, B. L. & BERRYMAN, A. A. 1977. Host resistance to the fir engraver beetle. 3. Lesion development and containment of infection by resistant *Abies grandis* inoculated with *Trichosporium symbioticum*. Can. J. Bot. *55*: 2358—2365.