ASPEN STAIN AND DECAY

WORKSHOP PROCEEDINGS

1995

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ABSTRACT

Proceedings of ASPEN STAIN & DECAY Workshop held Tuesday March 21, 1995 in Edmonton, Alberta. Contents include seven presentations supplied by the authors and a verbatim report of discussions that followed each talk. Recently published papers, associated with each topic are also included. The presentations have to do with a major program developed throughout the duration of the Canada-Alberta Partnership Agreement in Forestry, the investigation of Trembling Aspen stain and decay from a biological and utilization perspective.

ACKNOWLEDGEMENTS

Special thanks to L. Proehl for typing, arranging and formatting this report.

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Canadian Forest Service
(through the Canada-Alberta Partnership Agreement in Forestry)

Conference Steering Committee:

Y. Hiratsuka - Senior Scientist

D. Cheyne - Aspen Specialist

R.A. Bohning - Forest Products Technical Officer

TABLE OF CONTENTS

AGENDA

ASPEN STAIN & DECAY WORKSHOP- MARCH 21, 1995

NORTHERN FORESTRY CENTRE, EDMONTON, AB

9:00 am	Registration - Pine Room Foyer - Northern Forestry Centre Basement	
9:15 am	Welcoming Remarks	D. Cheyne, CFS
9:30 am	Aspen Decay & Stain Investigations In Alberta - Overview	Y. Hiratsuka- CFS
10:00 am	Impact of Decay & Stain on Mechanical Pulping	K. Hunt, PAPRICAN
10:30 am	Break	
11:00 am	Field Guide to Identify and Measure Aspen Decay and Stain	T. Stokes,Alberta Forest Service
11:30 am	Black Gall of Aspen and Incidence Of Decay	P. Crane, CFS
12:00 pm	Lunch - provided by Canada - Alberta Partnership Agreement in Forestry	
1:30 pm	Benzoic Acid, Salicylic Acid and Black Galls on Aspen	W.A. Ayer, University of Alberta
2:00 pm	Antagonistic Fungi of Aspen Decay & Stain and Their Potential Uses	P. Chakravarty, CFS
2:30 pm	Bioactive Metabolites of Antagonistic Fungi	L.S. Trifonov, University of Alberta
3:30 pm	End of Session	

Opening Remarks

Dave Cheyne welcomed everyone to the Northern Forestry Centre, and encouraged participants to talk with the researchers. After this, he provided some background.

The Canada-Alberta Partnership Agreement in Forestry (PAIF) undertakes programs of forest resource development and stewardship that emphasize the importance of the forest sector to the economic and social well-being of all Canadians. The "B-2" program manages projects involving forest products or processes utilizing hardwoods, small diameter trees, and mill residues.

One of the major programs in "B-2" and developed throughout the duration of the PAIF has been the investigation of trembling aspen stain and decay from a biological and utilization perspective. The information and material accumulated on this subject, by a team of experts led by Dr. Hiratsuka of the Canadian Forest Service (CFS), was presented at a one-day workshop held at the Northern Forestry Centre in Edmonton.

This report is a collection of the presentations made at the workshop and of the recently published papers associated with the research. Our aim is to provide an overview of the data on trembling aspen stain and decay. Some of the highlights of the work-to-date include the following:

* aspen pathogen identification system improved
* fibre protection initiated
* natural (clonal) properties/resistance in respect to stain and decay established
* aspen black gall and its decay inhibiting properties recognized
* aspen stain and decay field guide generated
* stain and decay pulp properties identified
* fibre hardness tester prototype developed.

The Aspen Field Guide, which was developed by CFS and Alberta Land and Forest Service personnel, will be available at the end of June, 1995. All participants will receive one.

Aspen decay and stain investigations in Alberta: an overview

Y. Hiratsuka

Canadian Forest Service, Edmonton, Alberta

Thank you, Dave. I appreciate the initiative shown by Dave Cheyne and Russel Bohning of Canadian Forest Service in organizing this workshop to review some parts of the accomplishments achieved with the funds provided through the B-2 committee of the Canada-Alberta Partnership Agreement in Forestry (C-A PAIF) initiatives and other funding agencies. These funds from other funding agencies have provided the backing for a significant amount of the research which will be presented here today.

The work started with C-A PAIF funds which was instrumental in attracting money from these other funding agencies (especially two NSERC Strategic Grants dealing with the micro-biological and chemical aspects of aspen decay and stain). Several industrial and provincial people in attendance today also provided their support to the grant applications. Personally, I would like to thank my superior, Dr. S. S. Malhotra, for encouraging me and supporting my involvement in this area of research.

All of you attending this workshop today know that, in recent years, aspen (*Populus tremuloides*) has become one of the most economically important forest tree species that occurs in boreal forest regions of Canada. The main use of aspen is pulping for paper-making. That is followed by particle board production and solid wood products (pallets, chopsticks, popsicle sticks, etc.). The harvest of this tree species has increased dramatically in recent years, but the economical utilization of this resource is often limited by the presence of extensive decay and stain caused by fungi and bacteria.

In 1983, I was asked to appear in front of a committee called the "Alberta Poplar Research Committee" which met in downtown Edmonton. I was told that poplar, especially aspen, was becoming the most important species in Alberta, and decay and stain seemed to be the important factors affecting the utilization of the wood. At that time, the main focus in my research was elsewhere. So, I reluctantly agreed to produce a report summarizing the type of information that was currently available and evaluate what would be the potential problem areas and opportunities for research. Together with a contract scientist, Dr. Loman, I published a small work entitled "Decay of Aspen and Balsam Poplar in Alberta" (see Y. Hiratsuka and A.A. Loman, 1984. Appendix 2). Since then, my co-workers and co-operators and I have been working on the subject. Today we are

going to present some of the results from our investigations related to the microbiological aspects of the work. The following were identified several years ago as the four major areas of emphasis in our work on aspen decay and stain:

1. Investigate the impact of decay and stain on aspen utilization especially for the pulp making process.
2. Compile a publication entitled "Field guide to classify and measure aspen decay and stain."
3. Explore the possibilities of biological or biorational control and protection of decay and stain.
4. Development of decay and stain resistant clones.

Each will be discussed further.

1. Investigate the impact of decay and stain on aspen utilization especially for pulping

Research on everything mentioned in this chart is still ongoing but, I would like to present here what we are doing and what level of understanding we attained. I would also like to discuss some of the directions the research will be taking. We have established an excellent cooperative research team. I hope that some of the industry people attending this meeting will be interested in aspects of our work and will help us carry out the practical applications. Here is a list of the cooperators in today's presentation:

Canadian Forest Service, Northern Forestry Centre

P. Chakravarty, P. Crane, P. J. Maruyama, L. Hutchison, K. Jakubec, and G. Herger.

University of Alberta, Department of Chemistry

W. A. Ayer, L.S. Trifonov, L. Browne

(Isolation, identification and bioassay of bioactive metabolites from fungi isolated in aspen.)

University of Alberta, Micro fungus Collection

L. Sigler

(Supported our project with the second largest fungal collection in Canada. Preservation of important fungal cultures and identification of fungi.)

University of Alberta, Department of Renewable Resources

B. Dancik, O. Rajora, F. Yeh (DNA fingerprinting of decay resistant clones.)

Alberta Forest Service (AFS)

D. Morgan, T. Stokes

Pulp and Paper Research Institute of Canada (PAPRICAN)

K. Hunt

Pulp quality testing.

Millar Western

G. Nielsen

Forest Engineering Research Institute of Canada (FERIC), Vancouver

J. Ewart

Developed H-gun

University of Calgary, Department of Biological Sciences

T. Thorpe

Development of tissue and cell culture methods.

2. Compile a publication entitled: "Field guide to classify and measure aspen decay and stain"

Traditionally, decay and stain are classified and measured in three categories: 1) advanced decay, 2) incipient decay, and 3) stain. Many people recognized that this classification is not reliable and causes a significant number of errors in classifying and measuring wood defects in aspen. After examining these defects and confirming microbial causes of each, we came up with a new classification of wood defects seen in aspen based on causal agents. This information was published in a report entitled, "Classification and Measurement of Aspen Decay and Stain in Alberta" (Y. Hiratsuka, D.A. Gibbard, O. Bakowsky, G. B. Maier. Information Report NOR-X-314, 29 pages, see Appendix 2).

Based on the feedback we received from field crews and provincial personnel, we are now preparing a publication entitled "Field Guide to Identify and Measure Aspen Decay and Stain" (see T. Stoke's (AFS) discussion). The field guide will be printed by the end of June, and it will be distributed to all of today's workshop participants.

As a part of the field guide, a hardness testing gun (H-gun), has been developed by FERIC engineers and a prototype of the gun is being tested. The H-gun is intended to distinguish between pulpable and unpulpable decayed wood. Ms. Stokes will be explaining and demonstrating the H-gun today.

3. Explore the possibilities of biological or biorational control and protection of decay and stain.

When wood chips are inoculated with a blue stain fungus there are problems in attaining the desired brightness in the CTMP pulping process.

More than one thousand cultures of fungi (representing more than 120 species) and several species of bacteria have been isolated from decayed and stained aspen wood as well as clean wood. Several of these fungi are antagonistic to decay fungi and stain fungi, and have been shown to protect aspen chips from blue stain fungi. Also, several novel antifungal compounds were discovered from these fungi by chemists at the University of Alberta. Microbial aspects of the work will be presented by Dr. Chakravarty. Chemical aspects of the work will be presented by Dr.Trifonov.

4. Development of decay and stain resistant clones.

When we started thinking seriously of the genetic improvement of aspen, it was important to consider disease resistance (including decay and stain resistance) along with fibre quality and growth characteristics. What we found is that levels of decay resistance is different from clone to clone suggesting genetic differences in the degree of decay resistance.

Aspen trees with black stem galls of unknown cause reportedly have less advanced decay caused by *Phellinus tremulae*. (See P. Crane's and W.A. Ayer's presentations.)

As you will witness through the various presentations today, I feel that we accomplished a great deal during the past ten years. I am particularly pleased that the excellent team of researchers and engineers in different disciplines could work together to attack the diverse problems of aspen decay and stain. However, as with most research, we found more questions and problems than clear answers and simple solutions. I would like to emphasize here that this is an excellent case of multi-disciplinary research and development work in action. CFS and AFS personnel cooperate very well. Excellent participation by Dr. K. Hunt (PAPRICAN), and J. Ewart (FERIC), has been significant. The participation of Dr. W.A. Ayer (University of Alberta) is especially significant because his laboratory is, probably, the most competent and productive natural product chemistry research team in the nation. I think we are very fortunate that Dr. Ayer's group put their efforts to tackling the identification of antifungal metabolites isolated from aspen microorganisms.

I believe that there are many interesting problems to tackle and solve in relation to aspen decay and stain. Some examples of future studies and opportunities are:

- Select and breed decay resistant aspen clones for future planting programmes.
- Conduct ecological investigation of major decay and stain fungi detrimental to pulp production. (How and where they colonize wood and which components of wood they consume, etc.).
- Increase experiments of chip inoculation with specific microorganism to evaluate their effect on pulp quality.
- Establish methods of application of antagonistic microorganism or antifungal metabolites in actual operational conditions, such as stored logs, chip piles, etc.
- Explore the possibilities of application of selected antagonistic fungi or antifungal metabolites to other forest products' situations such as, pulping of conifer species, protection of lumber products, etc.
- Market the H-gun.
- Develop decay detection methods applicable in the field (Ct Scan, Ultrasonic, etc.).

Every participant in this workshop, especially those from provincial government and industry, should critically evaluate what has been done so far and suggest what kinds of research will likely produce beneficial results to the industry in the long run. I believe that many excellent research and development opportunities exist and significant results will be generated if solid and sustained research support to the right projects are provided. It is essential that we find environmentally friendly solutions to deal with decay and stain problems in aspen.

For more information on aspen decay, see the attached papers in Appendix 2. Y. Hiratsuka and A.A. Loman. 1984. "Decay of Aspen and Balsam Poplar in Alberta." Information Report NOR-X-262, Northern Forest Research Centre, 19 pages and Y. Hiratsuka, D.A. Gibbard, O. Bakowsky, and G.B. Maier. 1990. " Classification and Measurement of Aspen Decay and Stain in Alberta." Information Report NOR-X-314 Northwest Region, 29 pages.

Impact of Decay and Stain on Mechanical Pulping

K. Hunt

PAPRICAN, Vancouver, B.C.

I'd like to thank Dr. Hiratsuka (CFS) for calling me up four years ago and asking me if I'd be interested in looking at the mechanical pulping of aspen. It just happened that, in the 1970's, even before it was very fashionable, I had been doing a lot of work on aspen, in particular, kraft pulping of decay and stained aspen wood cut in Ontario. So, this (Dr. Hiratsuka's suggestion) fit very well with what I was already doing.

I had a number of objectives when we first started. The main objective was to determine the effect of varying amounts of decay/stain in aspen wood on the amount of peroxide required to reach a defined brightness level for different mechanical pulps. In the shorter term we sought to find an answer to the following question: how would an increase of decay from 10 to 20% affect the amount of peroxide that would be needed to reach the defined brightness level, and how would this increase influence strength properties such as tear and tensile.

The major aspects of aspen that makes it so popular at the moment are as follows:

- It is very abundant here, in Alberta, with the largest volume of any place in Canada.
- It is the one species that occurs in every province in Canada.
- It has a low lignin content which is very good for chemical pulping.
- It's easy to propagate (cut it down and it comes back on its own).
- It is a very light coloured wood which is very important to mechanical pulping.

However, aspen can have a resin content that can cause pitch problems if logs or chips are not aged (especially for chemical pulping). Aspen stains and decays at a very early age. (Particularly if there is decay already in the roots when it starts to sucker).

Of the two major types of pulping, kraft pulping will very easily handle decay in aspen because colour is not the same problem as in the mechanical pulping process. The major problems you might run into in kraft pulping, if the amount of decay is increased, is that alkaline consumption will go up, and more extracted material will go to the recovery boiler. This extra material going to the recovery boiler may create a problem if the recovery boiler is overextended with processing the extra material.

In terms of mechanical pulping, we are doing very little chemical treatment of the wood and want to keep the brightness as high as possible. So, because stain and decay drop the brightness level, the amount of peroxide, or whatever brightening agent is used, must be increased. Problems can sometimes come later.

Because the lab pulping process may vary slightly from that at a mill, let's go through the three types we looked at and describe how we did them in the lab. The three types include Refined Mechanical Pulping (RMP), Chemithermomechanical Pulping (CTMP), and Alkaline Peroxide Refiner Mechanical Pulping (APRMP).

RMP is also known as open-discharge. With RMP, the chips enter the hopper and then pass into the eye of the refiner and out the bottom. It is all done under atmospheric pressure. The process may have to be repeated through this refiner to get the required freeness. Freeness is a measure of the drainability of the pulp. The lower the number, the less freely the pulp drains.

CTMP is done in a different apparatus. The screw-feed beneath the hopper has a three to one compression ratio. The chips which are broken down and compressed as they come out of the screw-feed, go through the chemical bath, which, in this particular case, is a mixture of sodium sulphite and sodium hydroxide where they absorb the liquor. The chips then go into a heating chamber where they are subjected to 20 psi pressure where they undergo an elevated temperature and pressure which are needed to soften up the lignin. The chips are carried by conveyor into the refiner and out through a relief valve. The whole process is completed under pressure. After this Thermomechanical pulp (TMP) process, the refined chip mix goes through the open discharge refiner to reduce it to the required freeness.

A two-stage alkaline peroxide refiner mechanical pulping (APRMP) uses the compression screw-feed part of the TMP process. In the APRMP process, material is first run through the compression screw feeder and then into a bath containing a sequestering agent. Peroxide is easily decomposed by metals which occur naturally in most wood. However, chips may also pick up various metal ions during the various processes: chipping, etc. Samples were taken from the sequestering process and stored until we were ready to start the rest of the operation. At that time, the material was put through the TMP screw-feed again. This time the bath consisted of peroxide, caustic magnesium sulphate, and silicate. After this bath, the material was removed and passed through the open discharge refiner unit.

Before we go on, let's briefly touch on the causes of decay (see Table 1). We looked at decay and stain caused by *Phellinus tremulae*, *Peniophora polygonia*, and *Ophiostoma crassivaginatum* (Types A, C, and E). The reason we left *Armillaria spp.* (Type B) out is that, being a root rot, it can be avoided by cutting off the bottom two metres and not using them. It can be noticed also that it is very dark wood in the middle. So, we felt that it would probably be very bad for pulping.

Table 1- Types and Causes of Decay and Stain

Type	Cause of Decay	Defect
A	*Phellinus tremulae* (most prevalent decay fungus of aspen)	Wood friable
B	*Armillaria spp.* root rot	Wood stringy
C	*Peniophora polygonia*	Wood firm
D	Variety of agents (fungi, bacteria, etc.)	Wood firm
E	*Ophiostoma crassivaginatum*	Wood firm (stain)

The variety of agents (Type D) was rather a catchall and not easy to find isolated samples. *Ophiostoma crassivaginatum* (Type E) was isolated at the Northern Forestry Centre.

Because Dr. Hiratsuka has already discussed Type A and Type C, I won't bother repeating what he has already said. The first set of samples that we had were taken from log yard piles. The resulting chips were not screened because of a time constraint. This simulated a worst case scenario. The amount of decay/stain was not adjusted so that it was down to the level seen in the mill (-15%). These were run through the RMP refiner. Type E sample was not available.

While plotting specific refining energy (the amount of energy put in per kilogram) versus the freeness (drainability), it was seen that the Type A sample, with the most decay, was the easiest to refine (see Figure 1). The sound wood results, represented by the open X on the graph, were very confusing. When we noticed that the less decayed Type A sample was about 50% harder to refine, I realized that we were probably dealing with different clones. Because of this, I couldn't really relate any of this evidence. So, Dr. Hiratsuka and Ms. Stokes went out and identified these clones by collecting decayed and sound wood. Then we repeated the RMP process on the samples. This time, everything fell in the same, straight, line (see Figure 2).

In Figure 2, U indicates an unscreened sample; S refers to screened material; the 15 indicates that Type A (*Phellinus tremulae*) had sufficient sound chip material added to get it down to 15% decay. This represents the worst case scenario for most mills.

I was surprised to find that Type A was not much lower in freeness than the sound wood. The only conclusion that I could come to was that the RMP refiner is not a particularly sensitive instrument. For the amount and severity of the decay present, it was not able to differentiate between them. Thus, they all are on the same line.

When I did the same thing with the CTMP refiner, the results were quite different (see Figure 3). Please note that the RMP values are higher so they take more energy. By adding chemicals, the amount of energy required to refine the samples is cut down. As would be expected, Type A, without any additional sound wood, was the easiest to refine. These data are illustrated as two lines, but there is no explanation why C15 (*Peniophora polygonia* - 15% decay) sample is higher. I suspect I should redraw these data as one curve and make this a sort of envelope which describes the overall scatter of the results.

Type A decay is being examined in terms of less refining energy demand. This can be explained in that we're dealing with different types of fungi which are doing various things, some enzymatically, some physically. Probably, the addition of alkali is promoting this great difference from the other decay types. In the case of Type A decay, some chemicals are physically getting in, so when we go to refine the chips it takes less energy.

When we look at the bound sulphur content, we see the sulphite treatment usually puts sulphonate groups into the pulp (see Table 2). The sound wood pulp has the largest content, followed by Types C and E. The least amount is in Type A. So, it looks like this fungus is modifying or eliminating the group "S" in the lignin and reacting with the sulphite more than the other fungi.

Figure 1 Freeness vs. Specific Energy

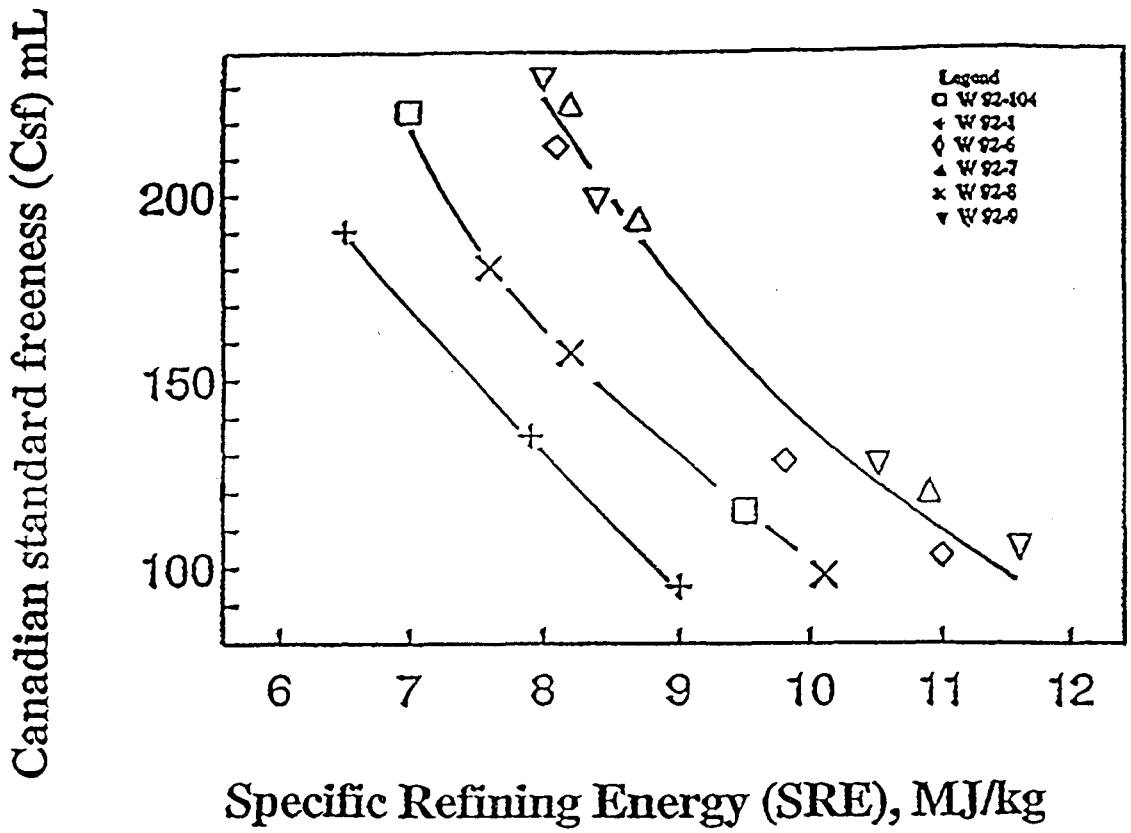

Figure 2 Freeness vs. Specific Refining Energy - RMP

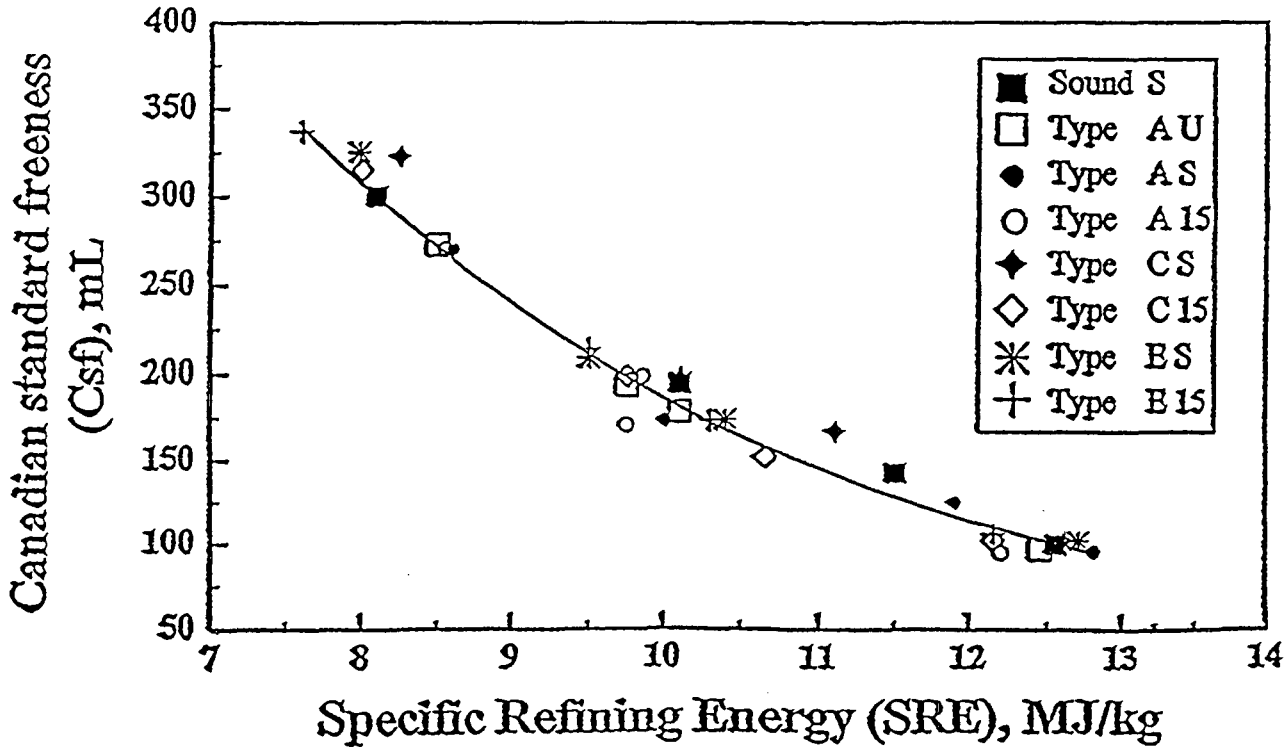

Figure 3 Freeness vs. Specific Refining Energy - CTMP

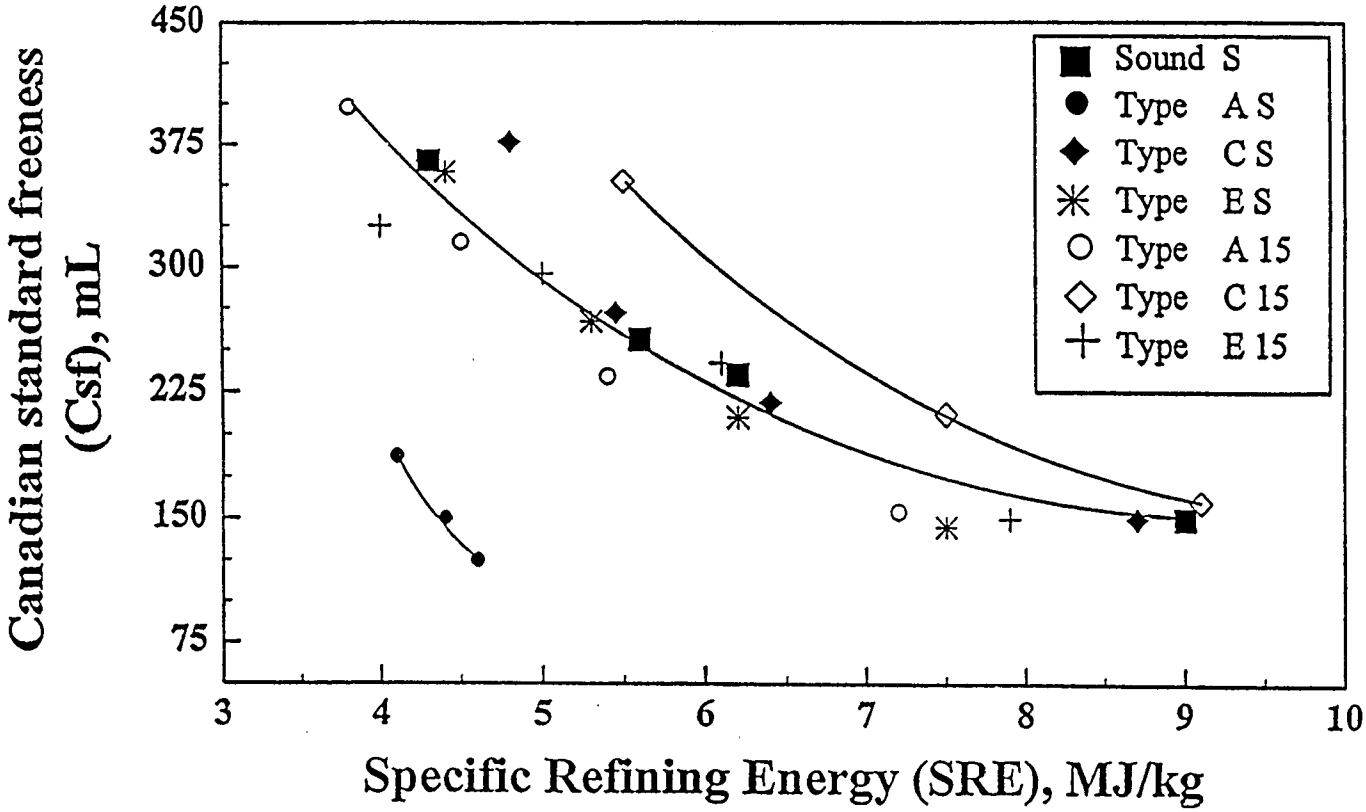

Figure 4 Brightness vs. Consumed H_20_2 - BCTMP

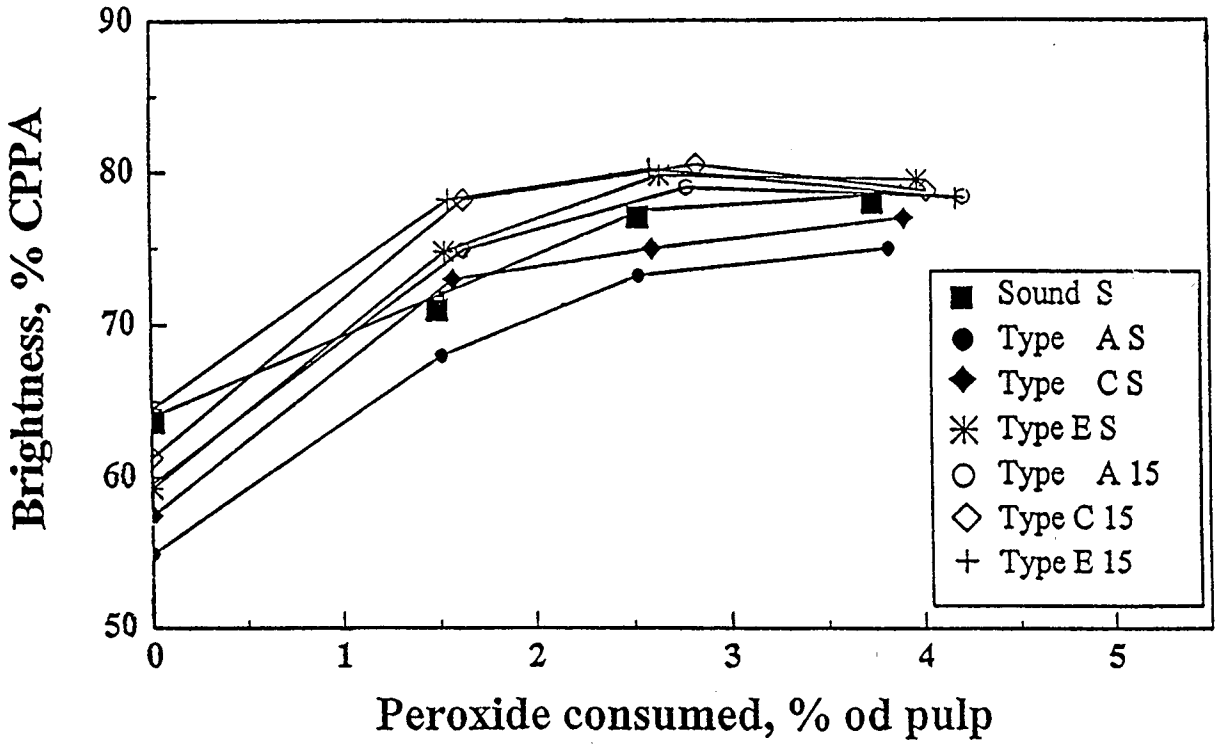

Table 2- Sulphur Content of Aspen CTMP Pulps

Sample	Bound Sulphur, % od pulp	Sulphonate Equiv., % od pulp
Sound S	0.110	0.275
Type A S	0.068	0.170
Type C S	0.086	0.215
Type E S	0.083	0.208

Brightness is one of the most important characteristics of mechanical pulping. To determine the bleachability of the pulp, we added different levels of peroxide: 2, 4, and 6 per cent (see Figure 4- Bleached Chemithermomechanical Pulp). Here, we're just plotting consumption. You can see that C and A samples that have the extra sound wood added tend to be much harder to brighten. Particularly this Type E (blue stain) was very hard to get rid of. You'll notice that for the sound wood and the ones with 15% decay, 80 brightness can be reached (see Figure 5- Bleached Refined Mechanical Pulp).

By virtue of the fact that chemicals have been added to the CTMP, a higher internal brightness in your pulp is obtained. As you can see here, it is, indeed, very hard to get above 80 brightness in the final stage. A considerable amount of peroxide or some other agent would have to be added if you wanted to get up to 85 brightness.

The original objective was to find out if there were differences in the amount of peroxide required to get the pulp to the same brightness level (see Table 3). You can see here the percentages of the decay used, and the amount of peroxide needed to get it to 78% CPPA brightness level. Actually, I wanted to get 80% brightness, but because we had problems with Type A pulp, it just seemed to level off and there was no way it would go up under these conditions. Even with Type C I had problems getting to 80% brightness.

Table 3- Peroxide Consumption of CTMP at Varying Amounts of Decay

Pulp	Decay %	CTMP pulp to 78% CPPA Brightness %, Peroxide Consumed, od pulp
Sound S	0	2.63
Type A S	51	ND
Type C S	40	4.33
Type E S	75	2.27
Type A 15	15	2.50
Type C 15	15	1.60
Type E 15	15	1.55

ND Not determinable.

In terms of the various optical and strength parameters for both bleached and unbleached samples, the brightness and the tear index differences between the sound and decayed wood were not that great. Within the unbleached, some are a little stronger, and some are a little weaker. Most of the values after bleaching have dropped below the value of the sound wood.

With tensile index (see Figure 6) almost all the values are above those of sound wood. Because of the better bonding between the fibres, the action of the various fungi on the wood may allow the refiner to defibrillate the individual fibres.

With scattering coefficient (see Figure 7) values were below the value of the sound wood.

With printing opacity (see Figure 8) values appear both above and below. But, none of our examples were really very far removed from the sound wood values.

Table 4- Specific Refining Energy & Peroxide Consumption: Aspen APRMP Pulp

Pulp	Csf, ml	Specific Refining MJ/kg	Initial ISO, % After Refining	Hydrogen Peroxide % od pulp, applied
Sound S	240	5.9	76.5	3.2
Type A S	210	3.7	74.0	3.2
Type C S	200	3.7	75.1	3.4
Type E S	205	3.7	75.9	3.0
Type A 15	203	4.1	77.7	3.5
Type C 15	232	4.2	76.3	3.1
Type E 15	231	3.9	76.7	3.1

We now look at the alkaline peroxide treatment (see Table 4), we tried to get a freeness in the range of about 200-250 ml Csf. Notice that the three decay types are around the same freeness and take the least energy to refine. The remaining pulp samples are of a higher freeness and energy consumption so if they were refined to the 200 ml Csf level, all the energy values would be even greater. Under the method we were using, it was hard to determine the exact amount of peroxide consumed. Future work will provide ideas about how to obtain the consumption figures accurately.

Following the refining stage, we did a further brightening stage with 85% brightness as our goal. We added the same amount of peroxide to each pulp and measured the resulting brightness (see Table 5). As before, the three undiluted decays gave the lowest brightness (all 81 to 83%) while the 15% counterparts were 83 to 85% brightness indicating that by optimization of the stages, 85% is achievable for all the samples. When compared to the bleached RMP pulp, the bleached APRMP pulps are of greater strength but, lower than for the bleached CTMP (see Table 6).

Figure 5 Brightness vs. Consumed H_2O_2 - BRMP

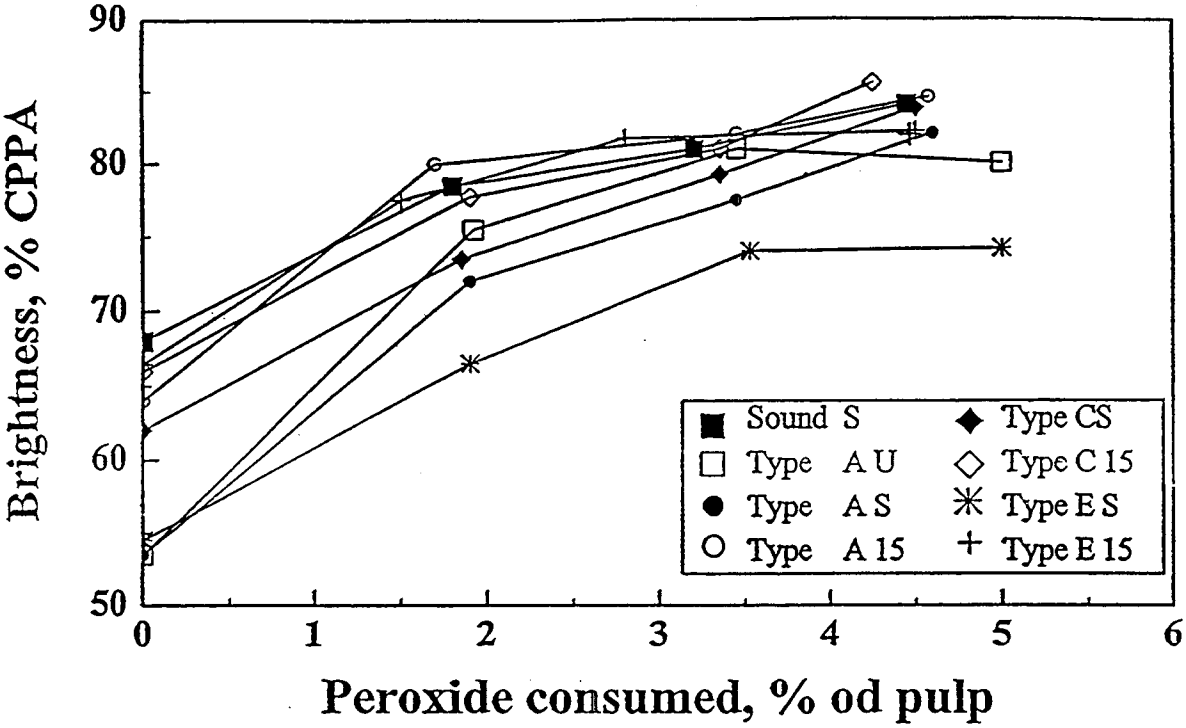

Figure 6 Tensile Strength vs. % Decay

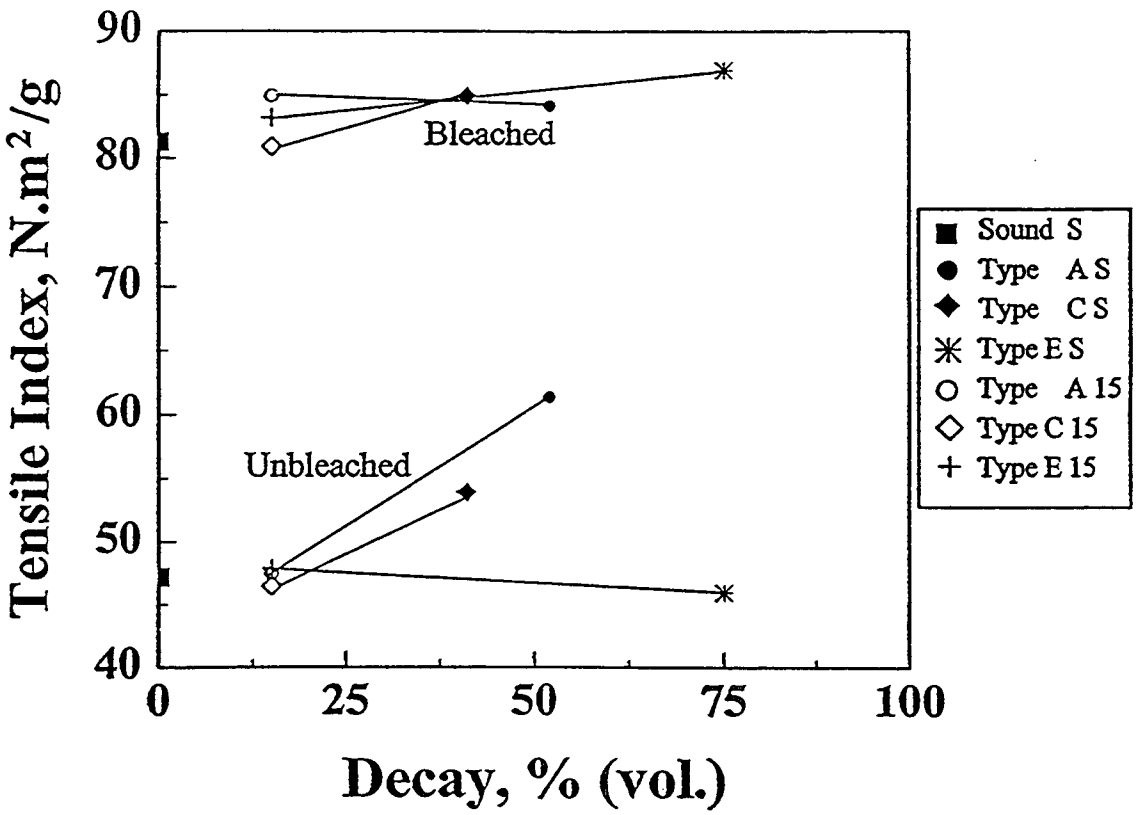

Figure 7 Light Scattering vs. % Decay

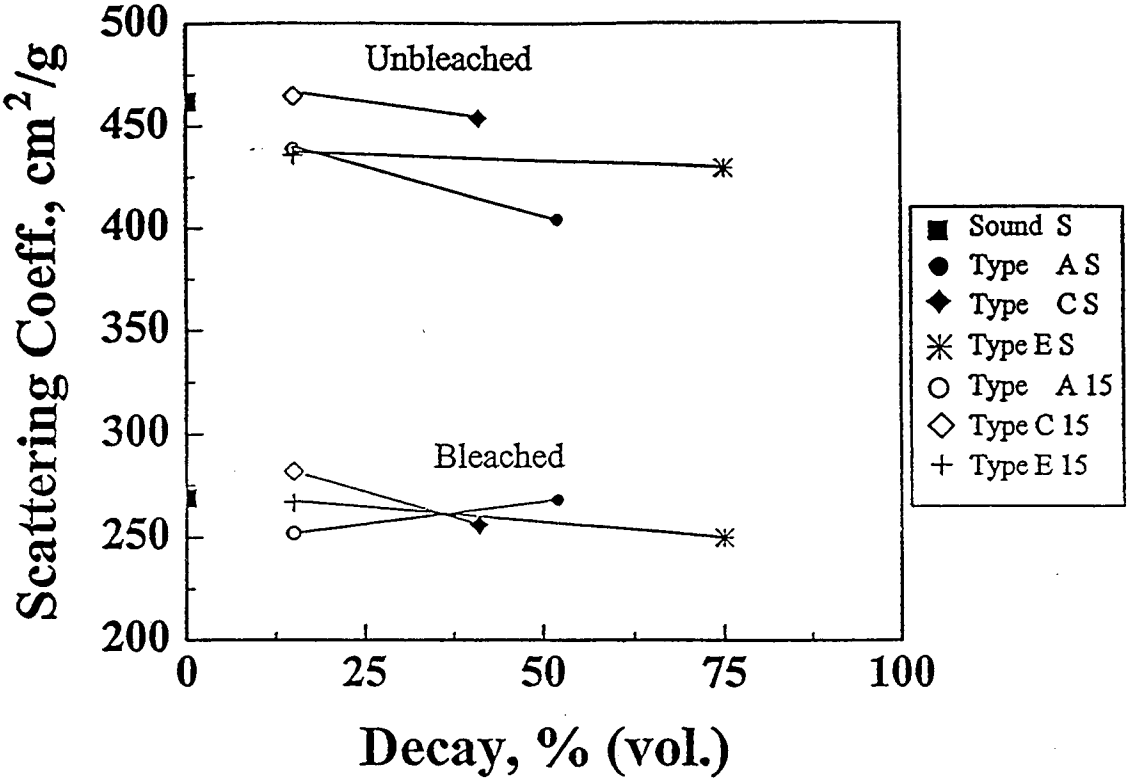

Figure 8 Printing Opacity vs. % Decay

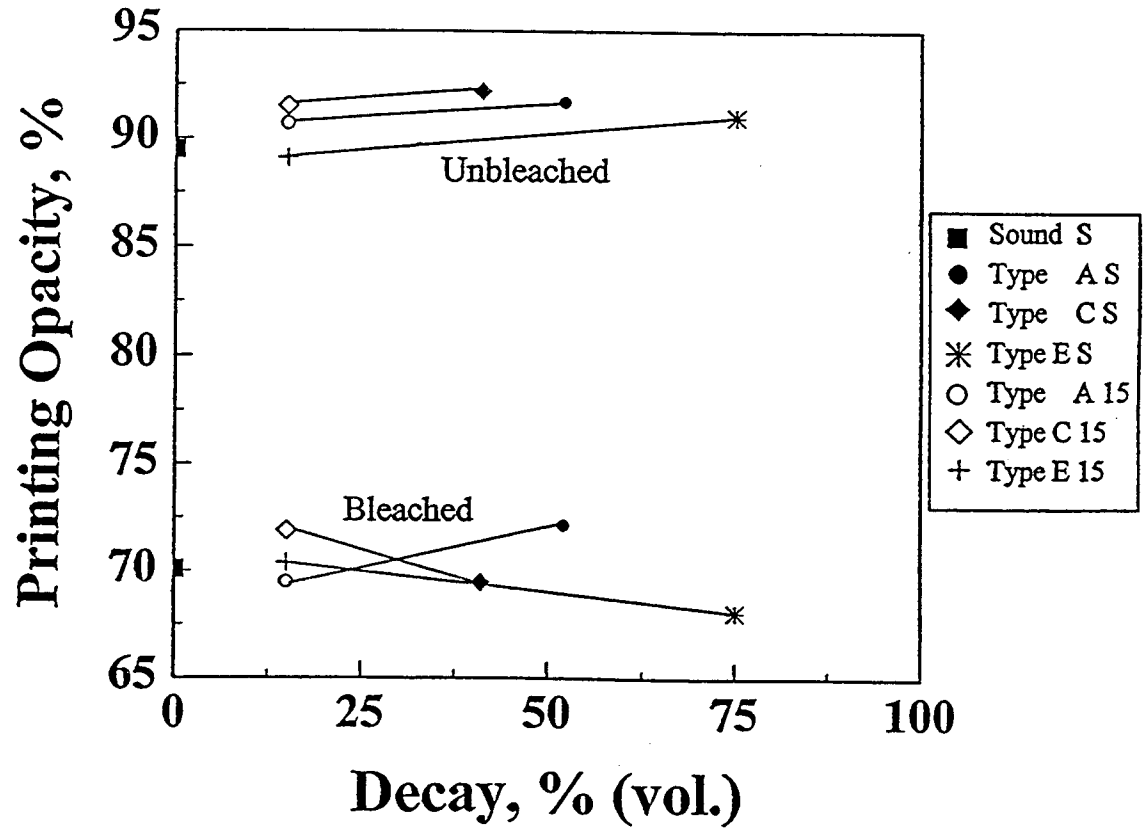

Table 5 Peroxide Consumptions and Final Brightness of APRMP Pulps

Pulp	Hydrogen Peroxide, % od pulp		Final ISO, %
	Applied	Consumed	
Sound S	5.0	1.53	84.2
Type A S	5.0	1.67	82.8
Type C S	5.0	1.98	81.4
Type E S	5.0	1.61	81.2
Type A 15	5.0	1.78	83.3
Type C 15	5.0	1.87	85.4
Type E 15	5.0	1.60	83.6

Table 6 Physical Properties of Brightened BAPRMP Pulps

Pulp	Bulk, cm/g	Printing Opacity, %	Tear Index, $mN.m^2/g$	Tensile Index, $N.m^2/g$	Scatter. Coeff., cm^2/g
Sound S	1.69	66.8	5.39	65.6	253
Type A S	1.68	62.9	4.80	78.8	203
Type C S	1.68	62.2	5.16	78.0	205
Type E S	1.68	60.8	5.49	70.8	193
Type A 15	1.61	61.0	5.51	74.8	196
Type C 15	1.76	68.2	5.13	69.0	260
Type E 15	1.71	64.5	5.02	78.9	216

Conclusion

- To be able to determine differences in pulps due to different decay types, the wood samples must be from the same clone or at least, two or more clones mixed at a fixed ratio.
- CTMP pulping process requires less specific refining energy to obtain the same freeness level as RMP.
- Bleached APRMP pulps are stronger than bleached RMP pulps, while bleached CTMP pulps are the strongest of the three types.
- No definitive factor could be found to relate an increase in decay with loss or gain in brightness, tear, or tensile strength.

Field Guide to Identify and Measure Aspen Decay and Stain

T. Stokes

Alberta Forest Service, Edmonton, Alberta

The first half of the field guide developed to identify and measure Aspen Decay and Stain and distributed to workshop participants under separate cover, deals with the visual aspects. This includes what the conk looks like, the expected size, and a general description of diseases that appear in aspen today. The last half of the guide describes the field procedures in making calculations to estimate cull and map and measure the decay found in trees.

Much of the recommended field methodology that is listed in the field guide is taken from the *Alberta Phase 3: Inventory Information, Tree Sectioning Manual* published by Alberta Land and Forest Services.

Land and Forest Services has been sectioning trees since the early 1960's and still use the same procedures today to collect data on our Permanent Sample Plot (PSP) programme. The procedures for tree sectioning are:

- Assess the tree for external indicators of decay; conks, scars, broken tops, and non-suspect blemishes.
- Mark the stump height and breast height.
- Fell the tree using standard felling techniques.
- Measure the tree length and mark off in 2.5 m sections starting at the stump.
- Take cross-sectional samples (cookies) at each mark, including the stump and breast height.

When the cookies are cut, note all decay and ascertain the extent of the decay along the stem. Then using a standard tree sectioning tally form, map the information (see Figure 1). Age the trees, measure for inside and outside diameters and sketch and measure any defect.

Figure 2 is an example of a sketch from a tree felled in PSP plot number 52. It is an aspen tree, 90 years old, from the Grande Prairie area, 28 metres tall, and 30 cm in diameter at breast height. I determined what type of decay is present by using pictures and guidelines from the field guide (see Table 1).

Figure 1- Tree Sectioning Tally Sheet

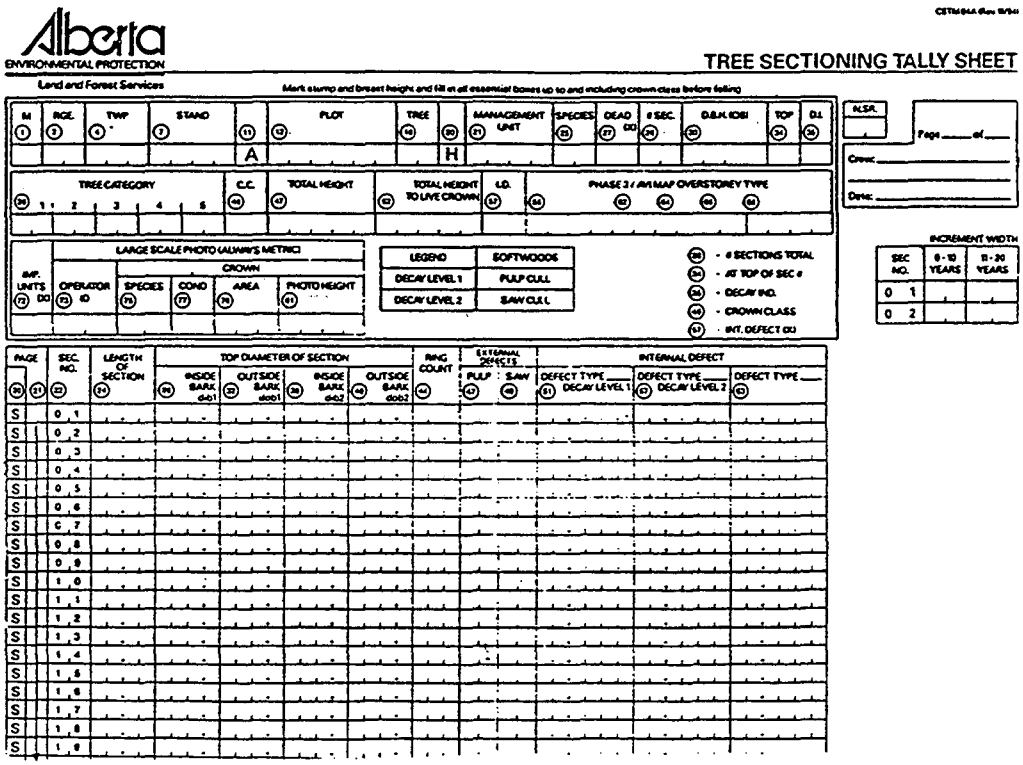

Figure 2- PSP #52 Grande Prairie

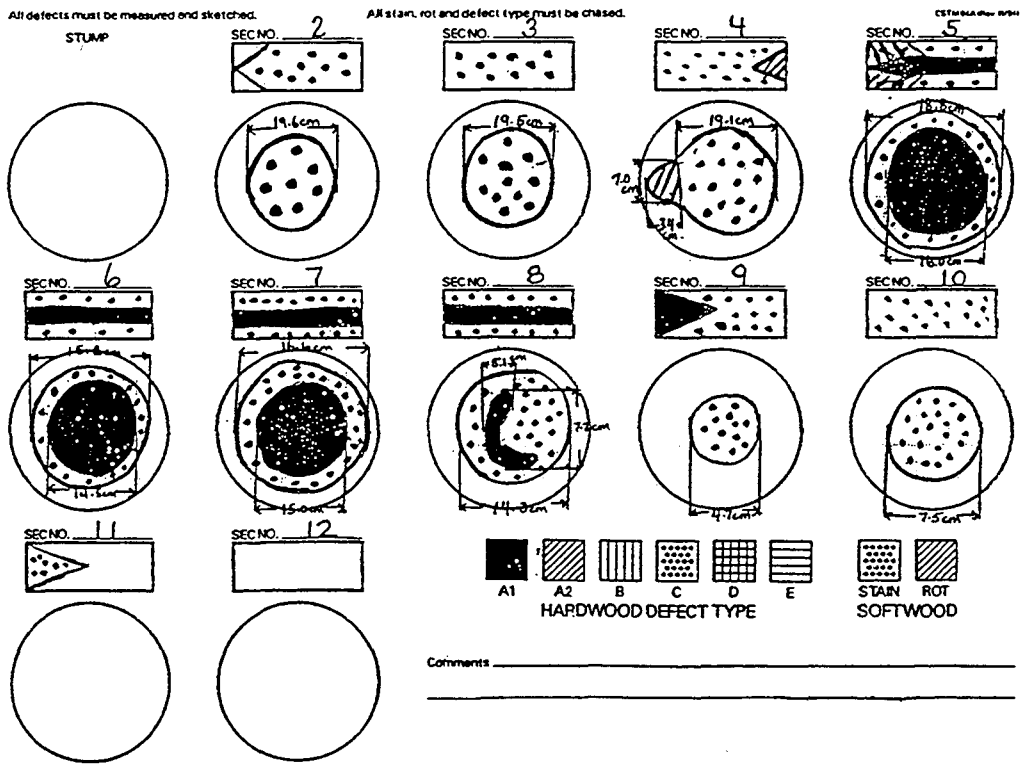

Table 1. Summary of the Defect Categories

Type of defect	Causes	Description of defect and external indicators	Defect distribution
A_1	*Phellinus tremulae*	White spongy rot bordered with black lines. Usually associated with conks. Decayed wood soft and unbleachable for pulping.	Usually occurs along most of the main stem
A_2	*Phellinus tremulae*	Decayed wood firm enough for pulping.	Often occurs along the main stem
B	Mostly *Armillaria* sp.	Yellow stringy rot often surrounded by dark brown fungal and wood material. Black shoestring-like fungal structures (rhizomorphs) present in and around the decay. *Armillaria* mushrooms may be present in the late summer or autumn.	Butt rot. Decay to about 1 m above the ground
C	Mostly *Peniophora polygonia*, occasionally *Radulodon americanus*	Stained column with irregular pockets of decay; pinkish to brownish. Often associated with fruiting bodies.	Along large portions of the main stem
D	Various causes (e.g., fungi, bacteria, abiotic factors)	Stain that does not reduce wood hardness.	Variable
E	Blue stain fungi	Grayish black sapwood stain.	On sapwood. Begins from cut ends or through damaged bark on fallen logs or logs stored in mill yards

These sketches of the decay and its extent are completed on the back of our tally sheets. The field guide comes in handy here. It helps to identify different types of stain and decay as they appear on a single tree.

The gross volume of the example tree was calculated. It is .8153 m^3. The total volume of Type A1 (non-pulpable) material for this tree is .1413 m^3. Based on the non-pulpable material, the net volume of this tree was calculated as .6741 m^3 or 82% pulpable material.

Based on the example tree, there are different levels of stain (i.e., level one pulpable stain). So we calculate all the volume, not just for one specific product such as pulp or OSB. The field guide is geared toward identifying stain and decay, and it is up to the users' interpretation to decide what type of fibre is present.

The last section of the field guide describes the use of the wood hardness tester otherwise referred to as the H-gun.

In 1990 a wood hardness testing device was created in order to remove the subjective estimates that usually resulted from using knives, fingernails, or pencils to determine the hardness of wood. Hiratsuka et al.[1] felt that a more objective means of determining the hardness of the wood should be found (see Appendix 2). Since the creation of the H-gun, a new prototype has been developed by FERIC in Vancouver.

The main change made to the old H-gun is a digital readout and a self-caulking handle where the needle retracts after each use. We would like to see the H-gun become a self-contained unit where the digital readout is placed on the handle.

The H-gun is proving to be a helpful tool in determining the transition between the non-pulpable *Phellinus tremulae* (Type A1) and the pulpable *Phellinus tremulae* (Type A2). We recommend that a relative rather than a definite H-gun reading be taken because the H-gun values of the same disk may vary. Moisture content and temperature conditions (frozen vs room temperature) of the disk affect the readings.

Overall, the field guide is designed to assist people in identifying the various types of decay and stain that exist in aspen. The latter part of the field guide briefly outlines an effective method of mapping and measuring decay in aspen. Researchers are currently assessing nondestructive methods of assessing decay in aspen. Their results have yet to be published.

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[1] Y. Hiratsuka, D.A. Gibbard, O. Bakowsky, and G.B. Maier. 1990. Classification and Measurement of Aspen Decay and Stain in Alberta. Information Report NOR-X-314 Northwest Region, 29 pages (see Appendix 2).

Black Gall of Aspen and Incidence of Decay

P. E. Crane

Canadian Forest Service, Edmonton, Alberta.

Several years ago, Dr. Hiratsuka made the chance observation that felled aspen trees with stem galls appeared to have sound undecayed wood even when quite old. He found decayed wood in only 1 of 20 large aspen trees with black galls, which is a much lower proportion than would be expected. This is quite remarkable, since earlier studies in the boreal forest regions of Alberta showed that decay occurs in 70% of the trees in 50 year-old stands. Many studies indicate that there is a pronounced relationship between decay and age or decay and diameter in aspen. A study was undertaken because there was a need for some basic information about black galls and their relationship to decay in aspen.

The work reported here was done at the Northern Forestry Centre while I was a graduate student in the Department of Plant Science at the University of Alberta.

What are black galls?

Very little is known about large stem galls on aspen. I found few reports in the literature, and even these few usually reported that the cause was unknown. I had two goals for this study: that it would provide basic information about black galls, and that it would form a basis for future studies of these structures. The study consisted of the following:

- Field surveys

  Is it true that gall trees are less likely to have extensive decay?

- Anatomy and histology

  What is the structure of galls? Clues to their formation or cause.

I will only touch on the second topic today, since it does not relate directly to decay incidence.

Black galls tend to occur in isolated groupings such as at Blue Ridge. There may be one stem gall on a tree or several. One tree had at least 10 main-stem galls. Some trees also had many branch galls but their structure indicated that these were probably caused by the same agent as the stem galls.

There appear to be at least two kinds of blackish stem galls on aspen. The first type of gall, which usually occurs on branches, has been found near Hinton, Whitecourt, and Chetwynd, B.C. They are caused by the poplar budgall mite. This tiny creature is only 1/5 mm long. It inserts its mouthparts into living cells of the host tree and induces it to produce deformed bud-like tissue. After several years, a distinct swelling develops on the tree. Even though galls normally grow only for a short time (2-4 years), we found mite galls that have been continuously occupied by mites for 80 years.

A second type of gall does not have deformed buds on the surface, but is more spherical and has large-thick bark projections. This type of gall formed the basis for both the field survey and the

detailed study of gall anatomy. We were not able to determine the cause of these galls. The wood is very hard and darkly stained with high amounts of lignin and gummy resinous substances.

- Normal sapwood: cells are straight; water-conducting vessels are wide and open; ray cells are living.
- Gall sapwood: many small, crowded cells; vessels are occluded by membranous structures; all cells contain extractives (gums, phenolics). Larger number of cells per growth ring forces them into unusual orientations, such as a whorl of cells.
- Macerated sapwood shows the deformities of all cells.

I would now like to discuss the results of our field survey to determine whether gall trees tend to have less decay than non-gall trees.

Methods

It was a challenge to find enough black galls to do a statistical survey. We were able to identify nine locations with at least eight gall trees in close proximity.

Field sites

- Elk Island National Park (3)
- Near Blue Ridge (4)
- Near Edson
- Near Drayton Valley

A single plot was established at each site to include all aspen trees with black galls and any interspersed or nearby trees without galls.

Decay indicator: Conks of *Phellinus tremulae*. This is the most reliable indicator of extensive internal decay. We did not consider stain and decay caused by other factors or organisms. All gall trees and adjacent non-gall trees were assessed for the presence of conks. Diameter was also taken for each tree. (Number of trees assessed: 529.)

Observations

These data were used to produce a regression equation to predict the odds of a tree having at least one decay conk. The factors considered were the presence or absence of a gall, the site, and the tree diameter. Using this model, there was good agreement between observed and expected values.

- Trees with galls were less likely to have conks than trees without galls.

  Odds of a gall tree having at least one conk was 44% of non-gall trees.

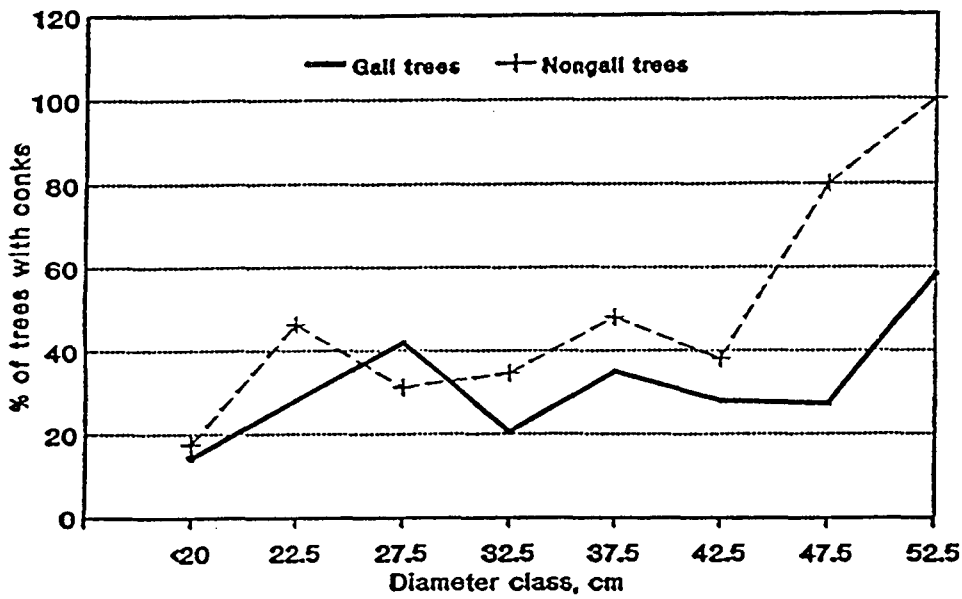
- Likelihood of conk formation varied with the site.

This probably relates to a difference among aspen clones in susceptibility to *Phellinus tremulae*. The Moss Lake site (Elk Island National Park) had the highest occurrence of trees with conks, whereas one of the Blue Ridge sites had the lowest.

- Likelihood of conks increased with stem diameter.

This is expected and has been previously shown[2]. In Figure 1 below, the trees are divided into diameter classes. A general increase in decay as trees grow larger is noted. This graph also demonstrates the general difference in percentage of decayed trees between gall and non-gall trees.

Figure 1. Percentage, by diameter class of gall and nongall trees with at least one *Phellinus tremulae* conk.



[2]G.B. Maier, and D. Darrah. 1989. Decay Levels in Mature Aspen Stands, Whitecourt, Alberta. Canada-Alberta Forest Resources Development Agreement, For. Can., North. For. Cent., Edmonton, Alberta.

Finally

Why should one tree stem have several conks, while a gall tree in close proximity is conk-free? There are several possible reasons for the observed decay reduction in trees with black galls.

Hypotheses of decay resistance

- Decay presence suppresses gall formation. (Might occur if *Phellinus tremulae* gets into a tree first and prevents colonization by a gall-causing organism.)
- Gall trees are genetically resistant to decay. (The observed decay reduction might occur if trees genetically resistant to decay are also prone to gall formation. The clusters of galls in small areas may indicate that the gall trees in a group are from one clone; however, this was not proven.)
- Causal organism produces protective chemicals. (Several fungi unique to black galls have been isolated and identified. One of these fungi has been shown to be antagonistic to *P. tremulae*. It is not yet known whether any of these fungi are important in the formation of black galls.)
- Tree produces protective chemicals in response to infection by a gall-producing organism.

This last possibility is being actively pursued by Dr. Ayer and his group of chemists at the University of Alberta, and he will report on their progress this afternoon.

Conclusion

Black galls are tumorous growths that have at least two causes.

This study has shown that there is less likelihood of extensive decay caused by *P. tremulae* in trees having black galls. In areas where black galls are common, it may be possible to more accurately predict decay levels. This study may also provide important background information for studies into decay resistance mechanisms in aspen. If we can understand the mechanisms of this decay resistance, new opportunities for reducing decay losses may be possible.

For more information on black gall in aspen see the attached papers in Appendix 3. P. E. Crane and Y. Hiratsuka. "Perennial Stem Galls of Aspen Caused by the Poplar Budgall Mite *Aceria parapopuli,*" Canadian Journal of Plant Pathology 16 (1992) :199-210 and P. E. Crane, P.V. Blenis and Y. Hiratsuka. "Black Stem Galls on Aspen and Their Relationship to Decay by *Phellinus tremulae,*" Canadian Journal of Forest Research 24 (1994) :2240-2243.

Discussion

Q. Were you able to determine if the cells were originally healthy ones?

A. I didn't look at the mite galls in detail, but only their general morphology. I looked at the detailed anatomy of the non-mite galls, including the cambial area and bark. It seems that there are an unusual number of cells produced right in the cambium and they are deformed.

Q. How confident are you that the mites actually cause the black galls?

A. This particular mite requires living tissue to survive. They secrete hormones or other substances into the tree that cause growths. So, if they were to come into contact with an older gall, I don't think that they could survive because there is such thick bark over the surface that I don't think they could get at the living tissue.

Q. So, do these mites move up and down the length of unaffected bark and penetrate the bark and create a gall?

A. Actually, there has to be some kind of crack or fissure for them to get into the living tissue.

Benzoic Acid, Salicylic Acid and Black Galls on Aspen

W.A. Ayer

University of Alberta, Edmonton Alberta.

I want to talk about a subject that needs no great introduction, black galls. In my group at the University of Alberta chemistry department, we have been working with some of the fungi associated with black galls as well as looking at the black gall itself. We have been making some progress in understanding the chemical composition of the galls, and in studying some of the antagonistic fungi that have been isolated here, at the Northern Forestry Centre (CFS). Dr. Trifonov will talk about those later on.

This paper outlines the chemical aspects of the galls. We have been working with the decay and stain fungi, the fungi from the black galls, and the chemistry of the black galls themselves.

Table 1- Decay and Stain of Aspen : Chemical Aspects

1. Decay and stain fungi
   Phellinus tremulae (heartwood rot)
   Peniophora polygonia (decay and stain)
   Ophiostoma crassivaginatum (blue stain)
   Ophiostoma piliferum (blue stain)

2. Fungi from black galls
   Phoma etheridgei (antagonistic to *P. tremulae)*
   Hyphozyma lignicola (antagonistic to *O. crassivaginatum*)
   Lecythophora hoffamannii (antagonistic to *P. t.* and *O. c.*)
   Sporormiella similis (antagonistic to *O. piliferum*)
   Stachybotris cylindrospora (antagonistic to *O. c.*)

3. Extraction of black galls

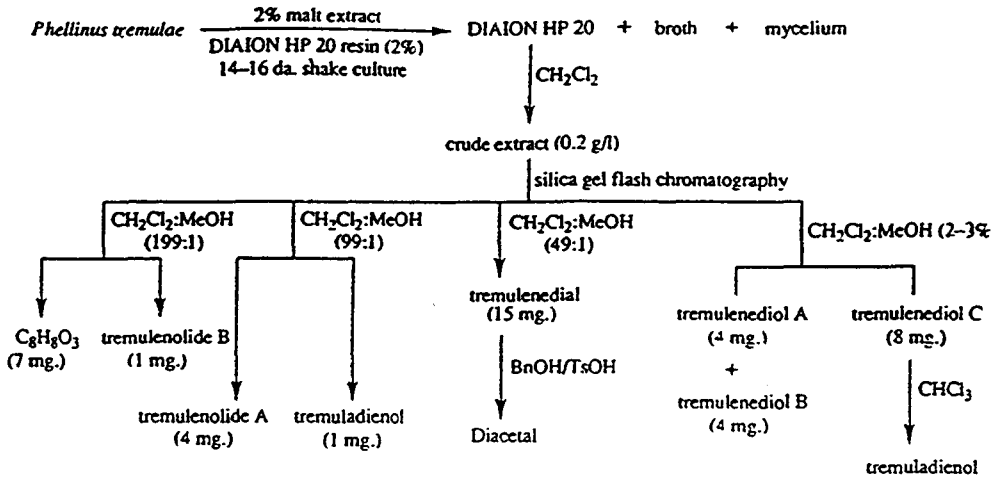
In collaboration with: Y. Hiratsuka, P. Crane, L. Hutchison, P. Chakravarty, and L. Sigler.

I will start by very briefly talking about some of the metabolites of *Phellinus tremulae*. As few structures as possible are shown.

This work was started on *Phellinus tremulae* by a graduate student, E. Cruz (University of Alberta). She looked at the metabolites produced by the fungus in liquid culture. She isolated a variety of compounds, the most important of which was a compound with the $C_8H_8O_3$ (see Table 2).

Ms Cruz was able to show that this was, in fact, 2-carbomethoxyoxepin, a compound that had never previously been isolated from natural sources. One of the contributions that Ms Cruz made was to show that if, when we grew the fungus, we added DIAION HP 20™ resin to the medium, it would

Table 2- Metabolites of *Phellinus tremulae* : Isolation



Methyl benzoate and methyl salicylate isolated by ether extraction of broth (GC–MS).

absorb the non-poplar compounds that were produced. That discovery greatly simplified the workup of the culture, and it contributed directly to the isolation of the very unstable, 2-carbomethoxyoxepin. This compound is the biogenetic precursor of salicylic acid or, in the case of *P. tremulae*, methyl salicylate, which gives a wintergreen odour to rotting aspen.

At the same time, we also isolated a new family of sesquiterpenes. These showed no activity against the wood rot fungi. We have yet to determine their purpose.

Next, G. Pausler, a postdoctoral student from New Zealand, was given the job of identifying what the black galls contain. First we had to collect some black galls. So, we went out into the field with Dr.Hiratsuka (CFS) and others to harvest some trees with black galls. We immediately chipped the chosen galls and then ground them to a powder. If not used immediately, they were kept frozen until we were ready to work with them.

We looked at three areas from the gall trees. Area A, the main gall itself; Area B, the border region, and clean wood from the gall tree, three to five metres away from the gall.

Because aspen contains many compounds, we decided to do some bioassays to see if we could find any activity. If we found no bioactivity, we would use what is known as a bioassay guided separation: no activity, no interest. I'm not going to discuss in any detail the extraction method we used. We wanted to find out what compound, produced in the black galls, inhibits the growth of *P. tremulae*.

The acidic components showed activity against *P. tremulae*. They (the acids) were separated into fractions; bioassays were carried out against *P. tremulae*, and then the active fractions were further purified.

Of the acids isolated, the only one that showed consistent bioactivity against *P. tremulae* was benzoic acid.

It is active at a range of about 800-1000 ppm against *P. tremulae* on carrot agar. Benzoic acid, which has many anti-fungal activities, is used commercially as an anti-fungal agent in preserves and jams. This seemed to be the active compound.

We went ahead and did the isolation first by preparative thin layer chromatography. In this way, we were able to show that 56 mg of benzoic acid was obtained from 40 g of black gall sawdust (see Table 3). That is a pretty good yield. Something in the order of one gram of benzoic acid per kilogram of the gall tissue. It also shows that the clean wood from the gall tree had nowhere near as much benzoic acid as the gall itself; however, it did have much more benzoic acid than what is in the clean wood of a clean tree.

Table 3- Quantitative Determination of Benzoic Acid

	Sample Size 40 g sawdust	benzoic acid	
•	Main gall	0.14% benzoic acid	(56 mg)
•	Clean wood, gall tree	0.012% benzoic acid	(5.3 mg)
•	Clean wood, clean tree	0.005% benzoic acid	(2 mg)

CH_2Cl_2 -- $NaHCO_3$ -- H^+ -- EtOAc -- PLC
Trees collected spring, 1993

Furthermore, we developed a more accurate procedure by using a more analytic process. We simply air-dried the sawdust and added p-toluic acid, not present in the wood, to have it serve as an internal standard for our extraction efficiency. Then we extracted with dichloromethane. We took the combined extracts and separated the acid fraction with sodium bicarbonate and then we re-acidified and re-isolated the acids. The dichloromethane extract was concentrated with phenylacetic acid, because phenylacetic acid was not present in the wood but, was used for an internal standard for the efficiency of our gas chromatography equipment. In this way we have been fairly confident of the levels of benzoic acid present in the various tissues.

Table 4 shows an analysis of benzoic acid levels in the black gall tissue. Black gall tissue contains about 1700 ppm of benzoic acid. The clean wood from the gall tree contains about 90 ppm of benzoic acid, and the clean wood from the healthy tree contains about 14 ppm. We also analyzed clean wood from *P. tremulae* infected trees which showed they contained about 39 ppm of benzoic acid.

Table 4- Analysis of Benzoic Acid in Aspen.

	Source	%Benzoic acid	Benzoic acid ppm
•	black gall tissue	0.17	1700
•	clean wood, gall tree	0.0090	90
•	clean wood, healthy tree	0.0014	14
•	clean wood, *P. tremulae* infected tree	0.0039	39

The question that arose at this point was: is it benzoic acid that is causing the black gall effect, or is it benzoic acid which is protecting the gall bearing trees from attack by the fungi? This is where the work Ms Cruz was doing began to overlap with the work of Dr. Pausler.

In the biosynthesis of 2-carbomethoxyoxepin, the first thing that happened when benzoic acid came into contact with *P. tremulae* was that it became methylated. *Phellinus* species are very good methylating fungi. It was taking the benzoic acid out of the way, cleaning it out, in the form of the inactive methyl benzoate. The methyl benzoate was then oxidized by an oxygenase to give, in the case of the methyl ester, methyl salicylate, and in the unmethylated case, salicylic acid.

In recent years, it has been shown that salicylic acid plays a key role in systemic acquired resistance (SAR) in many plants. Salicylic acid is one of the oldest known natural products. It was isolated first in 1828. Its acetylated form, acetylsalicylic acid (ASA or aspirin) is the most widely used pharmaceutical preparation in humans.

About ten years ago people began to wonder why plants make salicylic acid. The answer is that it is a key agent in fighting pathogens when they invade plants. Not only is salicylic acid important in human diseases, it is also important to the plants that produce it to combat pathogens. The pathogen activates enzymes which then transform benzoic acid into salicylic acid.

We came to the position where we could suggest that the benzoic acid, itself, was protecting the galled trees. Or, that the presence of large amounts of benzoic acid made readily available large amounts of salicylic acid which then were signal molecules in alerting plant defenses. At present, we have these two hypotheses, and we don't know if one, both, or neither are correct.

That brings you pretty well up to date with our research. However, in talking to Dr. Hiratsuka (CFS) and Ms. Stokes (Alberta Environment Protection, AEP) we decided that some clones of aspen seemed to be less susceptible to attack by *P. tremulae* than other clones. So, with Ms. Stokes and help from the AEP, we were able to collect a number of different clones to test.

G. Herger (University of Alberta) has been looking at the benzoic acid and salicylic acid levels in the different clones of aspen we collected (see Table 5). It is, however, our impression that there is not really a great clonal difference.

Table 5- Comparison of benzoic acid (BA) and Salicylic acid (SA) levels in three different clones of aspen

Extract	BA (ppm) 1st extraction	SA (ppm)	Height
4-1-CTA	7.81	1.04	2 m
4-1-CTB	4.21	1.09	3.2 m
4-1-CTC	8.85	1.73	4.2 m
5-2-CTA	4.82	1.17	1.5 m
5-2-CTB	4.08	3.89	5 m
5-3-CTC	9.97	1.53	10 m
5-3-CT	1.17	0	
5-3-LCT	7.38	1.28	
6-1-CTA	15.16	0.1	0.5 m
6-1-CTB	9.36	0	2 m
6-1-CTC	6.15	0	10 m

I'll acknowledge my co-workers here. From the chemistry department: E. R. Cruz, L. D. Jimenez, G. Pausler, L.S. Trifonov, A.Q. Khan, and S. Miao. The people at the Canadian Forest Service: Y. Hiratsuka, P. Chakravarty, L. Hutchison, P. Crane, and G. Herger. L. Sigler of the University of Alberta Microfungus Herbarium.

For more information on benzoic acid and aspen interactions see the attached paper in Appendix 4. M.G.Pausler, W.A.Ayer, and Y.Hiratsuka. "Benzoic Acid, Salicylic Acid and Black Galls on Aspen," 1995, 17 pages.

Antagonistic Fungi of Aspen Decay and Stain and Their Potential Uses

P. Chakravarty

Canadian Forest Service, Edmonton, Alberta.

In recent years aspen (*Populus tremuloides Michx.*) has become increasingly important as a source of pulp for the paper-making industry. Decay and stain caused by various fungi have been identified as the two most important factors limiting the utilization of aspen. The economic consequences of the types and degree of decay and stain differ significantly according to the end use. More than 250 species of fungi have been associated with decay in aspen and many other non-decay or endophytic fungi have been isolated from decayed, stained, and clear aspen wood.

Three major decay fungi in aspen are recognized. These include *Phellinus tremulae* Bond. et Bor., *Armillaria* spp., and *Peniophora polygonia* (Pers.:Fr.) Bou. et Galzin. *Ph. tremulae* is the most serious wood destroying fungus and it causes columns of trunk decay. The affected wood becomes soft, spongy, and not good for pulping. The second most common cause of decay in aspen is *Pe. polygonia*. This fungus does not cause large columns of advanced decay as does *Ph. tremulae*, but it is more often found in decayed and discoloured wood than *Ph. tremulae*. The decay caused by *Pe. polygonia* is acceptable for the pulp and paper making industry, and requires less bleaching than does wood decayed by *Ph. tremulae*. Species of *Armillaria* cause butt or root rot in aspen. This type of decay occurs only at the base of the tree and tapers off less than two metres above the ground.

Table 1- Effect of Culture Filtrate of *Pe. polygonia* on the *in vitro* Growth of *Ph. tremulae*

Inoculation period (weeks)	Mycelial dry wt. (mg) of *P. tremulae*					
	Control			Culture filtrate of *Pe. polygonia*		
	ME	PDB	CE	ME	PDB	CE
1	15.5c	30.0b	49.9a	14.5c	30.5b	48.7a
2	20.0e	55.0c	75.a	22.5e	48.3d	68.4d
3	45.6d	70.0b	96.0a	38.0e	61.5c	70.9d
4	62.5e	85.5c	108.3a	49.5f	69.5d	71.0b

Values are the means of 20 replicates. Means followed by the same letters in a row are not significantly (P=0.05) different from each other by Scheffe's test.
ME=malt extract, PDB=potato dextrose broth, CE=carrot extract.

Species of *Ophiostoma*, *Ceratocystis*, and *Verticicladiella* cause blue or sap stain in harvested logs or in wood chips. Blue stain is a major problem since discoloured wood chips reduce the quality of the end product. The presence of stain in more than trace amounts is not acceptable in furniture,

molding, and other specialty products because of the reduction of the aesthetics. During pulping, increased bleaching is required to obtain the required brightness level when infected chips are used.

In our laboratory, we have been studying the interactions of various decay, non-decay, and blue stain fungi and their potential uses in biological control.

Interactions of *Ph. tremulae* and *Pe. polygonia*:

Pe. polygonia inhibited the *in vitro* growth of *Ph. tremulae* when grown in dual culture. The presence of a culture of *Pe. polygonia* reduced the growth of *Ph. tremulae* in a volatile metabolite test. A culture filtrate of *Pe. polygonia* reduced the growth of *Ph. tremulae* in liquid culture (Table 1). On aspen wood blocks, the growth of both the fungi was inhibited, and they formed a dark brown line at their contact point. Chemical analysis of the culture filtrate showed presence of ten volatile and sixteen non-volatile compounds. Among these, two volatile and three non-volatile compounds significantly inhibited *in vitro* growth of *Ph. tremulae*.

Interactions of *Phoma etheridgei* and *Ph. tremulae*:

P. etheridgei, isolated only from black gall of aspen, produced antifungal compounds that were strongly inhibitory *in vitro* to *Ph .tremulae* on agar, in liquid media, and on aspen wood chips, and may play a role in the natural ecosystem as an antagonist to *Ph. tremulae.*

Decay caused by *Ph. tremulae* is a serious one. Our results, indicate that both *Pe. polygonia* and *P. etheridgei* could be considered as potential candidates for biological control agents against *Ph. tremulae.*

Interactions of blue stain or sap stain fungi and endophytic fungi:

Three endophytic fungi, *Stachybotrys cylindrospora* C.N. Jensen, *Lecythophora hoffmannii* (van Beyma) W. Gams & McGinnis, and *Sporormiella similis* Khan & Cain were found to be strongly antagonistic to two species of blue stain fungi, *Ophiostoma piliferum* (Fr.) H. & P. Sydow and *O. crassivaginatum* (H.D. Griffin) T.C. Harrington. The *in vitro* growth of *O. piliferum* and *O. crassivaginatum* was reduced in dual culture on agar media and in liquid culture when treated with a culture filtrate of these endophytic fungi. Both *O. piliferum* and *O. crassivaginatum* failed to colonize aspen wood chips when they were pre-inoculated with these endophytic fungi. A number of compounds were isolated and identified from the culture filtrates of these endophytic fungi. These compounds showed varied fungitoxic effect against *O. piliferum* and *O. crassivaginatum* (Table 2 and 3).

Table 2- Effect of Compounds Produced by *Stachybotrys cylindrospora* on the *in vitro* Growth of *Ophiostoma piliferum*

Compounds produced by *S. cylindrospora*	Reduction of growth of *O. piliferum* from control (%) 1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml
2-Methyl butanoic acid	0.0a	17.5b	46.5c	100.0d
Isovaleric acid	0.0a	42.5b	100.0c	100.0c
Isobutyric acid	0.0a	18.7b	100.0c	100.0c
Maltol	0.0a	0.0a	0.0a	0.0a
Sodium phenyl acetate	0.0a	0.0a	0.0a	100.0b
Sodium isovalerate	0.0a	0.0a	0.0a	100.0b
Sodium 2-methyl butyrate	0.0a	0.0a	42.5b	100.0c
Sodium isobutyrate	0.0a	0.0a	0.0a	100.0b
Phenylacetic acid	0.0a	11.3b	46.5c	100.0d
p-Hydroxy benzoic acid	0.0a	18.7b	100.0c	100.0c
2-Phenyl ethanol	0.0a	25.0b	42.5c	100.0d
Anthranilic acid	0.0a	11.3b	100.0c	100.0c
Tiglic acid	25.0b	75.0c	100.0c	100.0c
3,3-Dimethyl acrylic acid	50.0c	100.0d	100.0c	100.0c

Values in each row followed by the same letter are not significantly (P=0.05) different from each other by Scheffe's test.

Table 3- Effect of Compounds Produced by *Stachybotrys cylindrospora* on the *in vitro* Growth of *Ophiostoma crassivaginatum*

Concentration (µg/ml)	% reduction of growth of *O. crassivaginatum* from control	
	Trichodermin	Trichodermol
1	0.0c	0.0c
10	26.7c	13.4b
100	100.0a	100.0a
1000	100.0a	100.0a

Values in each row followed by the same letters do not differ significantly (P=0.05)

The blue stain fungi reduce the quality of the resulting pulp and paper especially in the chemithermomechanical pulping process. Chlorinated phenols were used in Canada for years to prevent sap stain and mold growth on unseasoned lumber and wood chips. These compounds are no longer used because of their toxicity and persistence in the environment. Our results indicate that three endophytic fungi could be considered as potential replacements for these chemicals to serve as biological protection agents against blue stain fungi on aspen. We will be conducting large-scale inoculation trials of these endophytic fungi on aspen wood chips to confirm their action against blue stain fungi and to determine their effect on the pulping process.

For a more complete understanding of antagonistic fungi of aspen decay and stain see journal articles, P. Chakravarty and Y. Hiratsuka. "Antagonism of Two Decay Fungi, *Peniophora polygonia* and *Phellinus tremulae* Associated with *Populus tremuloides,*" European Journal of Forest Pathology 22 (1992): 354-361 and P. Chakravarty, L.S. Trifonov, L. Hutchison, Y. Hiratsuka and W.A. Ayer. "Role of *Sporormiella similis* as a Potential Bioprotectant of *Populus tremuloides* Wood Against the Blue-stain Fungus *Ophiostoma piliferum,*" Canadian Journal of Forest Research 24(1) (1994): 2235-2239, in Appendix 5.

Discussion

Q. What is the black line seen in wood infected with *Phellinus tremulae*? Is it dead wood?

A. It is not dead wood, it is the pigment that is produced by the fungus.

Bioactive Metabolites from Antagonistic Aspen Fungi

L. S. Trifonov

University of Alberta, Edmonton Alberta.

The chemical approach to aspen decay and stain was based on the antagonism observed in dual cultures between fungi associated with aspen. The fungus inhibiting the growth of other fungi was grown in liquid or solid medium, and the metabolites isolated from the mycelium and from the culture broth were studied. This paper is the result of these studies.

Peniophora polygonia is the second most common cause of aspen decay. In a recent study it was found that *P. polygonia* is antagonistic to the major aspen decay fungus *Phellinus tremulae*. The objective of the chemical investigation was to isolate and identify the metabolites produced by *P. polygonia* in liquid culture and examine their possible inhibitory effect on the growth of *P. tremulae*. The metabolites that were isolated fall into the following groups: sesquiterpenes, aromatic compounds, and volatiles.

Seven new sesquiterpenes having the drimane skeleton were isolated. The aromatic compounds are either substituted benzaldehydes or aryl propanediols. Of all these compounds only two benzaldehydes and the new sesquiterpene peniopholide showed inhibition at 1000 ppm.

At this stage it was obvious that the active compounds should be somewhere else. Accordingly, the volatile metabolites were analyzed by means of gas chromatography (GC), GC-IR, and GC-MS. Most of them showed activity higher than the aromatics and peniopholide. Finally, a structural analog of isovaleric acid, 4-phenyl-3-butenoic acid, known as an inhibitor of the enzyme peptidylglycine hydroxylating monooxygenase - was tested. It was determined that this compound inhibits the growth of *P. tremulae* at a concentration as low as 1 μg/ml (6μM) (see Table 1).

A study of the structure-activity relationship was extended and many analogs (synthetic and commercial) of the inhibitor were tested. Some of them were shown to be even more active than the enzyme inhibitor.

Phoma etheridgei is a new species described by Hutchison and Hiratsuka. It often occurs on the surface of black galls of aspen in Alberta, and is strongly antagonistic to *P. tremulae* and *Ophiostoma crassivaginata*. For a long time the problem with this fungus was the inconsistent results obtained by using mycelium for inoculation. Spore suspension was found to give satisfactory results. Five closely related phenolic metabolites were isolated of which only one, phomalone, showed 100% inhibition of *P. tremulae* at a concentration of 1mM (ca. 250 ppm).

Table 1: Volatile metabolites of *Peniophora polygonia*

Compound	Liquid Medium V8 Juice	Inhibition of *P. tremulae* Concentration (µg/ml)			
	%	1	10	100	1000
Isobutanol	10.0	-	-	-	-
Isoamyl alcohol	16.1	-	-	-	-
2-methylbutanol	9.7	-	-	-	-
Isobutyric acid	9.2	-	-	+	+++
Isovaleric acid	2.3	-	-	-	+++
2-Methylbutanoic acid	0.3	-	-	+	+++
Benzaldehyde	19.0	-	-	++	+++
2-Phenylethanol	3.3	-	-	++	+++
3-Phenylpropanol	2.4	-	-	++	+++
Benzoic acid	2.4	-	-	++	+++
Unidentified	26.3	-	+	++	+++
2-Phenylbutanoic acid		-	-	+	+++
4-Phenyl-3-butenoic acid		+	+++	+++	+++

- : no activity; + : 25% inhibition; ++ : 50% inhibition; +++ : no growth

Phialophora alba has been isolated frequently from aspen which is not infected with other fungi. The possibility that this fungus might protect aspen from attack by other fungi prompted us to investigate its metabolites and to test their activity against aspen decay causing fungi.

Several anthraquinone, as well as one unusual chlorine-containing hydroxyanthrone were isolated, characterized, and tested. Two compounds showed 50% inhibition of the growth of *P. tremulae* at a concentration of mg/ml (1000 ppm).

Lecythophora hoffmannii turned out to be the most challenging fungus. Although it strongly inhibited the growth of the two major stain-fungi, the results from liquid cultures were inconsistent and the isolation, the purification, and the structure determination were very difficult. More consistent results were obtained when the fungus was grown on solid rice as a medium. Only one biologically active compound was isolated from rice culture. It was only a few weeks ago when the structure of this metabolite was determined by Dr. N. Kawahara as the monosodium salt of the sulfate of a previously described metabolite of another fungus (*Monochaetia dimorphospora*). This unique fungal metabolite showed very strong activity - 100% inhibition of *O. crassivaginatum* and 50% inhibition of *Ophiostoma piliferum* at 10 ppm.

Stachybotrys cylindrospora is known as an endophytic fungus on aspen. Three groups of metabolites were isolated from the liquid medium of this fungus and their structure solved: a) stachybotridial and stachybotramid; b) a substituted isochromane - all three inactive but new natural products, and c) two known trichothecene mycotoxins - trichodermin and trichodermol which were shown to be responsible for the strong inhibition of the blue stain fungus *O. crassivaginatum* (20% inhibition at 10 ppm and 100% inhibition at 100 ppm). Unfortunately, these two compounds are toxic to humans, which could compromise the use of *S. cylindrospora* as a bioprotectant.

Sporormiella similis is a strong candidate for biological protection. It is considered primarily to be a coprophilous (dung) fungus but, was consistently recovered from the bark of the black galls. It showed very strong inhibition of the blue stain fungus *O. piliferum*. Ten metabolites were isolated by chromatography or identified by GC combined with other spectroscopic methods. Several of them were found to be quite active *in vitro* at a concentration as low as 1 ppm, which makes them potential candidates for chemical protection.

Ophiostoma (Ceratocystiopsis) crassivaginatum is a typical blue stain causing fungus growing on aspen. The objective of this investigation was to answer the question, "What is causing the black colour of wood infected by this fungus?" Accordingly, we were looking for $FeCl_3$ - positive compounds, which could form dark complexes with iron in nature. A large number of $FeCl_3$ - positive phenolic compounds were isolated, which are considered, at least in part, responsible for the discolouration of the wood infected by this fungus.

For a more complete review of bioactive metabolites from antagonistic aspen fungi, see L.S. Trifonov, P. Chakravarty, Y. Hiratsuka and W.A. Ayer. "Antifungal Activity of Metabolites of *Peniophora polygonia* Against the Aspen Decay Fungus *Phellinus tremulae*," European Journal of Forest Pathology 22 (1992) pp 441-448, in Appendix 6.

Appendix 1

Appendix 1

Aspen Stain & Decay Workshop - Attendees' List

Last Name	First Name	Organization
Anderson	Ken	Alberta Newsprint Company
Anderson	Robert	Land & Forest Service-Rocky/Clearwater
Ayer	William	University of Alberta
Bella	Imre	Canadian Forest Service- NoFC
Blenis	Peter	University of Alberta
Bohning	Russel	Canadian Forest Service- NoFC
Chakravarty	Priyotosh	Canadian Forest Service- NoFC
Clark	Jean	Forintek Canada Corp.
Crane	Patricia	Canadian Forest Service- NoFC
Darrah	Dale	Land & Forest Service- Whitecourt
Gauthier	Kyle	Alberta Pacific Forest Industries Inc.
Greenway	Ken	Alberta Environmental Centre

Last Name	First Name	Organization
Harman	David	Mistik Management Ltd.
Hee	Margarete	Land & Forest Service- Whitecourt
Herger	Gabriele	University of Alberta
Hunt	Kenneth	PAPRICAN
Hiratsuka	Yasuyuki	Canadian Forest Service- NoFC
Ip	David	Tree Plan Canada- CFS-NoFC
Lakusta	Tom	Land & Forest Service- Edmonton
MacDonald	Ronald	Technology Brokers Inc.
Malhotra	Surj	Canadian Forest Service- NoFC
Mallett	Ken	Canadian Forest Service- NoFC
McKinnon	Joe	Weldwood Canada
Morgan	Dave	Land & Forest Service- Edmonton
Muir	Dave	University of Alberta
Nielsen	Gregg	Millar Western Pulp (Whitecourt) Ltd.

Last Name	First Name	Organization
Niemi	Florence	Daishowa-Marubeni International Ltd.
Osterhout	Barb	Land & Forest Service- Edmonton
Ouellet	Richard	Alberta Pacific Forest Industries Inc.
Rajora	Om	University of Alberta
Sanders	Gordon	Slave Lake Pulp Corporation
Sieusahai	Permanand	Slave Lake Pulp Corporation
Stokes	Teresa	Land & Forest Service- Edmonton
Thomas	Barbara	University of Alberta
Trifonov	Latchezar	University of Alberta
Witham	John	Land & Forest Service- Whitecourt
Williamson	Grant	Ainsworth Lumber- Grande Prairie

Appendix 2

DECAY OF ASPEN AND BALSAM POPLAR IN ALBERTA

Y. Hiratsuka and A.A.Loman[1]

INFORMATION REPORT NOR-X-262

NORTHERN FOREST RESEARCH CENTRE
CANADIAN FORESTRY SERVICE
ENVIRONMENT CANADA
1984

[1] Loman and Associates, 4915 Vanguard Road N.W., Calgary Alberta T3A 0R5

Cover photos:

Left: Vertical section of an aspen stem with fruiting body of *Phellinus tremulae* and a column of advanced decay. Right: Cross section of an aspen stem with advanced decay caused by *Phellinus tremulae*.

Hiratsuka, Y.; Loman, A.A. 1984. *Decay of aspen and balsam poplar in Alberta*. Environ. Can., Can. For. Serv., North. For. Res. Cent., Edmonton, Alberta. Inf. Rep. NOR-X-262.

ABSTRACT

Aspen (*Populus tremuloides* Michx.) and balsam poplar (*P. balsamifera* L.) represent about 40% of Alberta's forest resources, but utilization is only 1% of the total available. Decay and stain are two major factors limiting utilization of these species. Major decay-causing agents and types of decay of aspen and balsam poplar are reviewed, and the implications for management and utilization are discussed.

RESUME

Le peuplier faux-tremble (*Populus tremuloides* Michx.) et le peuplier baumier (*P. balsamifera* L.) constituent environ 40% des ressources forestières de l'Alberta, mais leur utilisation ne s'élève qu'à 1% du total disponible. Les deux principaux facteurs limitant leur utilisation sont la carie et les taches colorées. Les principaux agents et les types de caries observés chez ces deux essences sont discutés, de même que leurs répercussions sur l'aménagement et l'utilisation de ces essences.

CONTENTS

FIGURES

TABLES

INTRODUCTION

Hardwoods, mostly aspen (*Populus tremuloides* Michx.) and balsam poplar (*P. balsamifera* L.), represent about 40% of Alberta's forest resources. Current utilization amounts to only about 1% of the total, but the time is fast approaching when the softwood resources will be harvested to their limits of annual allowable cut. The aspen-balsam poplar resource will then be expected to play a much more significant role in meeting the demand for forest products in the future. The relative amounts of hardwood (mostly poplar) volume across Canada and potential annual allowable cut in Alberta are shown in Figures 1 and 2. Typical aspen forests in Alberta are illustrated in Figures 3 and 4. Three other poplar species (black cottonwood, *P. trichocarpa* Torr. & Gray; eastern cottonwood, *P. deltoides* Bartr.; and narrowleaf cottonwood, *P. angustifolia* James) also occur in Alberta, but they are economically insignificant.

Decay and stain have been identified as two of the most important factors limiting the utilization of aspen and balsam poplar. Several terms used in this report are defined as follows: Incipient decay indicates an early stage of decay in which the wood is hard and firm but may show discoloration or dark lines. Advanced decay refers to an advanced stage of decay of wood that is softened and has lost structural strength. Stain is a discoloration of wood by microoganisms or other physiological causes and may or may not be the early stage of incipient decay.

Many reports containing information on decay of aspen and balsam poplar have been published in various parts of North America, including Alberta. The most significant and relevant contributions are the following: Alberta (Bailey and Dobie 1977; Paul and Etheridge 1958; Thomas et al. 1960), Manitoba and Saskatchewan (Wall 1971; Black and Kristapovich 1954), Ontario (Basham 1958, 1979; Riley 1952), Colorado (Davidson et al. 1959; Hinds and Wengert 1977), and Minnesota (Meinecke 1929). Unfortunately, the above-mentioned reports and many others written on the subject are not easily accessible because they have been reported mostly in specialized scientific journals or unpublished government reports.

The purpose of this report is to compile and review available information on decay of aspen and balsam poplar and to attempt to interpret it in relation to aspen-poplar management and utilization in Alberta.

This report was prepared originally for the Alberta Poplar Research Committee, composed jointly of personnel from the Alberta Forest Service (J. Soos, B. Karaim, and E. Gillespie) and Blue Ridge Lumber (1981) Ltd. (M. Summers, P. Caldwell, and R. Kerr) and chaired by A.D. Kiil of the Canadian Forestry Service.

MAJOR DECAY-CAUSING AGENTS AND TYPES OF DECAY

Many fungi have been isolated and identified from decay and stain of aspen and balsam poplar (Basham 1958; Gilbertson 1981; Hinds and Wengert 1977; Lindsey and Gilbertson 1978; Thomas et al. 1960; Wall 1971). Fourteen major decay fungi have been isolated and identified from incipient and advanced decay in aspen and balsam poplar in the Slave Lake area of Alberta by Thomas et al. (1960) (Table 1). According to Thomas et al., *Phellinus tremulae* (=*Fomes igniarius*) is the most common and important decay-causing organism in both aspen and balsam poplar. This species has also been identified as the main decay organism of aspen in other parts of North America (Basham 1958; Black and Kristapovich 1954; Hinds and Wengert 1977; Meinecke 1929; Wall 1971). This fungus is commonly called the false tinder fungus and causes soft yellowish and characteristic brown to blackish brown lines surrounding each decay column (Figs. 5-7). Hoof-shaped sporophores (conks) of *P. tremulae* are about 10 cm in width (Fig. 8). The upper side of the sporophore is greyish black at first and later becomes cracked and appears cinder-like. The undersurface is covered with brown to whitish-brown spore-producing tubes. The interior of a conk is dark brown (Fig. 5).

On aspen, *Radulum casearium* and *Peniophora polygonia* (=*Corticium polygonium*) have been commonly and constantly isolated from yellowish or reddish stringy rot and from variously colored (brown, yellow, red) incipient decays. They are considered to be the next most important aspen decay after *P. tremulae* (Basham 1958; Black and Kristapovitch 1954; Laflamme and Lortie 1973; Thomas et al. 1960; Wall 1971).

Characteristically, *P. polygonia* has been isolated from younger aspen trees, and most isolations were from incipient decay (Basham 1958). Sporophores of *P. polygonia* are often observed on branch stubs of trees

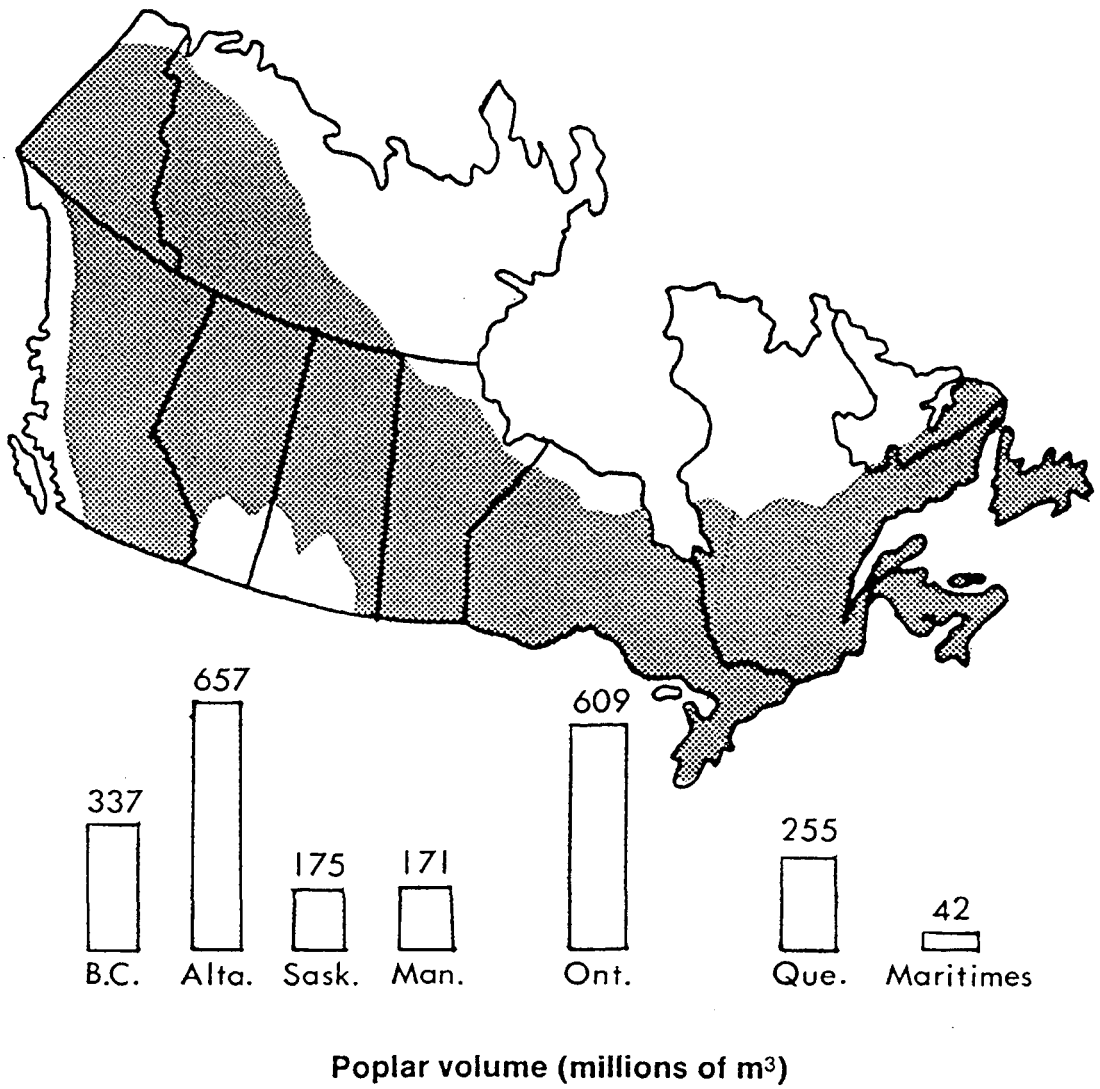


Figure 1. Poplar distribution across Canada (shaded area) and estimated gross merchantable volume for each province or region (based on Bonnor 1982).

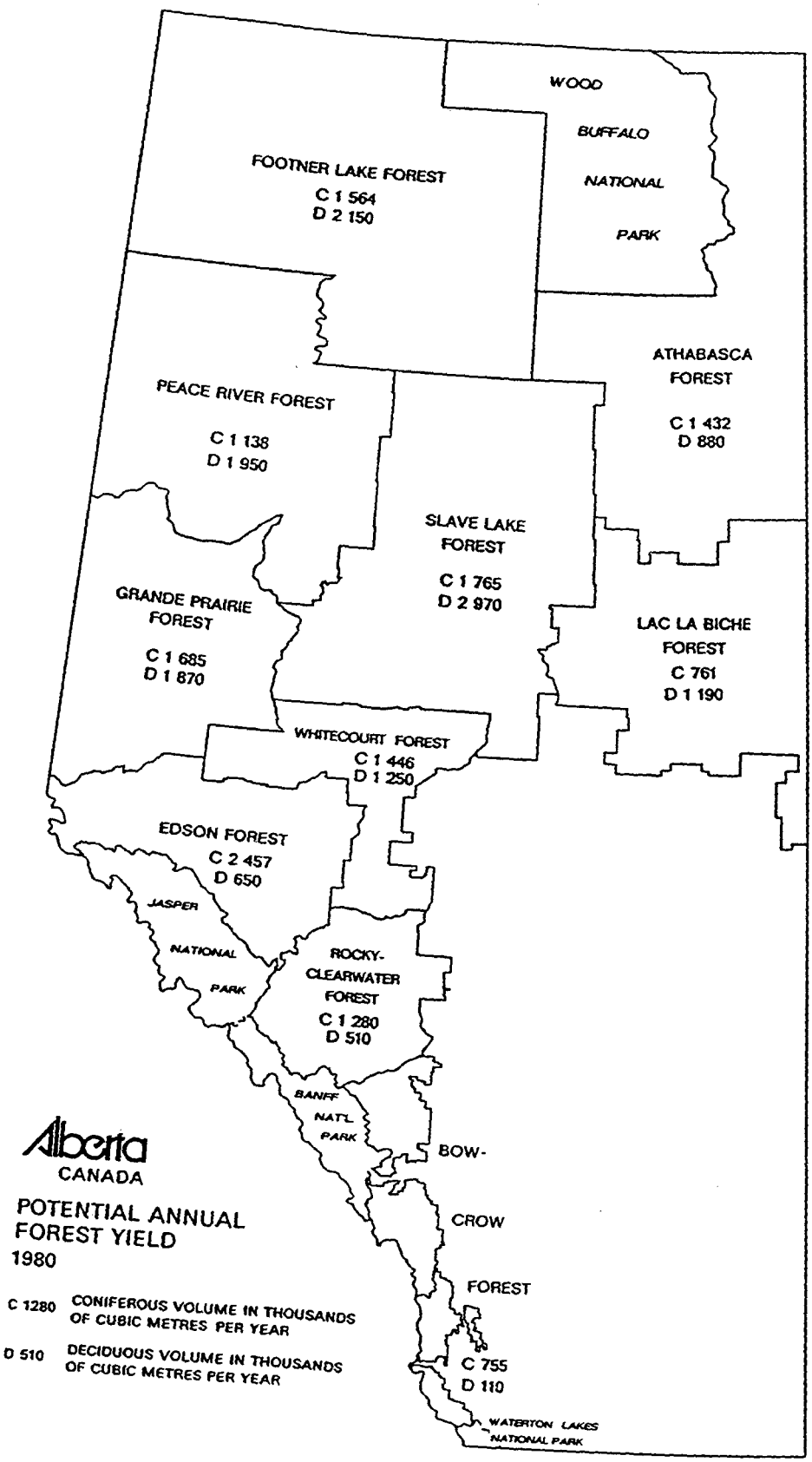


Figure 2. Potential annual forest yield of coniferous and deciduous (mostly poplar) species in Alberta. (Alberta Forest Service map.)

Figure 3. Pure aspen stand along a logging road near Blue Ridge, Alberta. Figure 4. A stand of mature aspen near Blue Ridge, Alberta, after softwood harvest.

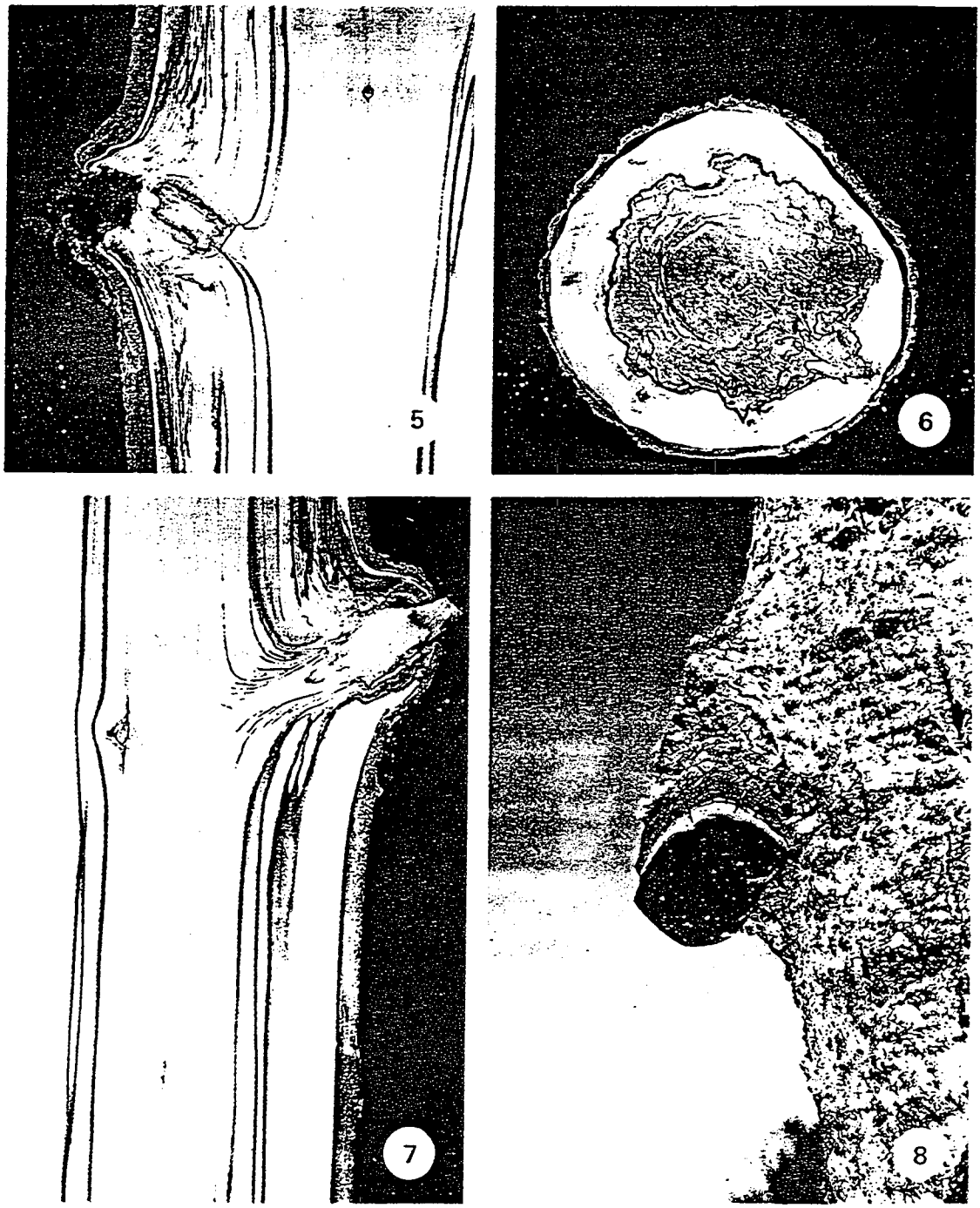


Figure 5. Vertical section of an aspen stem with a fruiting body of *Phellinus tremulae* and a column of advanced decay bordered by characteristic black lines. Figure 6. Cross section of an aspen stem with advanced decay caused by *Phellinus tremulae*. Figure 7. Relation of rotten knot and decay column of *Phellinus tremulae* in aspen. Figure 8. A fruiting body (conk) of *Phellinus tremulae* on aspen.

Table 1. Percentage infections by major decay-causing fungi on aspen and balsam poplar in the Slave Lake area of Alberta. Based on Thomas et al. (1960).

	Aspen		Balsam poplar	
	Trunk	Butt	Trunk	Butt
Phellinus tremulae (=*Fomes igniarius*)	13.4	0.2	26.8	—
Radulum casearium	14.2	1.2	—	—
Peniophora polygonia (=*Corticium polygonium*)	12.8	1.5	—	—
Coriolus zonatus (=*Polyporus zonatus*)	2.1	trace	—	—
Bjerkandra adusta (=*Polyporus adustus*)	1.5	0.7	2.5	0.1
Pholiota adiposa	0.5	0.2	—	—
Phlebia strigosa-zonata	—	2.2	—	—
Armillaria mellea (=*Armillariella mellea*)	—	0.9	trace	1.6
Gymnopilus spectabilis (=*Pholiota spectabilis*)	0.1	1.2	1.3	4.2
Pholiota destruens	—	—	17.7	0.4
Corticium expallens	—	—	5.2	0.4
Trechispora raduloides	0.5	trace	0.8	0.1
Corticium vellerum	—	—	0.3	0.1
Pholiota subsquarrosa	—	0.4	—	—
Mixed	12.4	0.7	—	—
Unknown	10.7	1.4	34.7	3.5
Total	89.2	10.8	89.5	10.5

of small dimensions (Fig. 9) or on old stem scars (Fig. 10). The wood supporting sporophore-bearing branch stubs or stem scars is characteristically in a state of yellowish and pinkish incipient decay (Fig. 11). *Peniophora polygonia* is seldom associated with advanced decay.

Although *R. casearium* is commonly isolated from living trees, sporophores are usually found only on dead trees. They are resupinate, yellow-brown, and have coarse teeth. Basham (1958) suggested that *R. casearium* may be the cause of most of the characteristic advanced stringy trunk rot of aspen.

Of the three most dominant decay fungi on living trees mentioned above, only *R. casearium* is commonly isolated from stored log piles, indicating that the vitality of the other two species decreases in storage (Fritz 1954).

On balsam poplar, *P. tremulae* is again recognized as the most common and dominant decay-causing species, followed by *Pholiota destruens* (Fig. 12), *Corticium expallens*, and *Bjerkandra adusta* (=*Polyporus adustus*) from trunk rot and *Gymnopilus spectabilis* (=*Pholiota spectabilis*) and *Armillaria mellea* from butt infections (Figs. 13, 14) (Thomas et

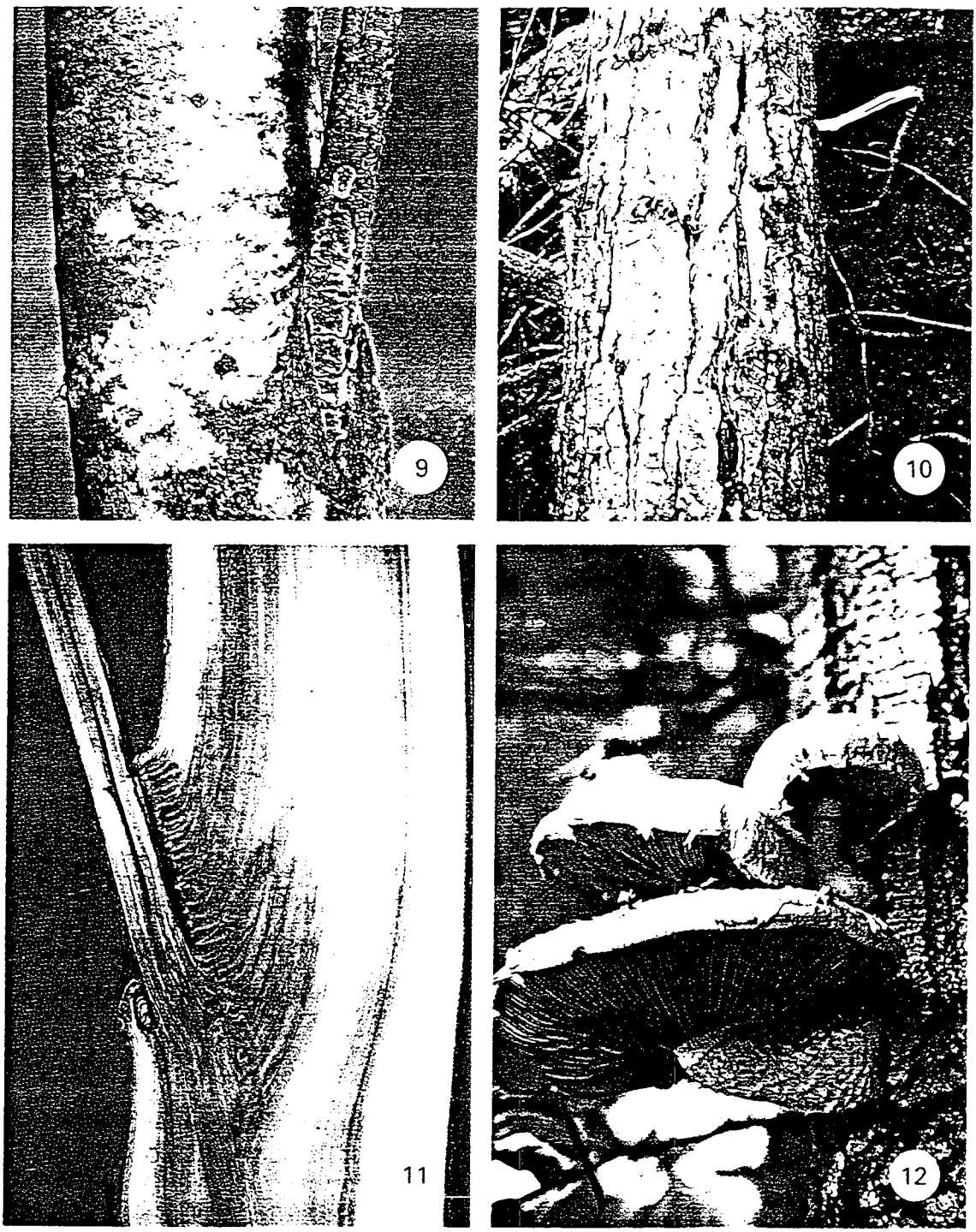


Figure 9. Fruiting structure of *Peniophora polygonia* on aspen. Figure 10. Large scars (cracks) on mature balsam poplar. Figure 11. Dead branch and column of discolored incipient decay of aspen caused by *Peniophora polygonia*. Inside of Figure 9. Figure 12. Fruiting bodies (mushrooms) of *Pholiota destruens* on balsam poplar.

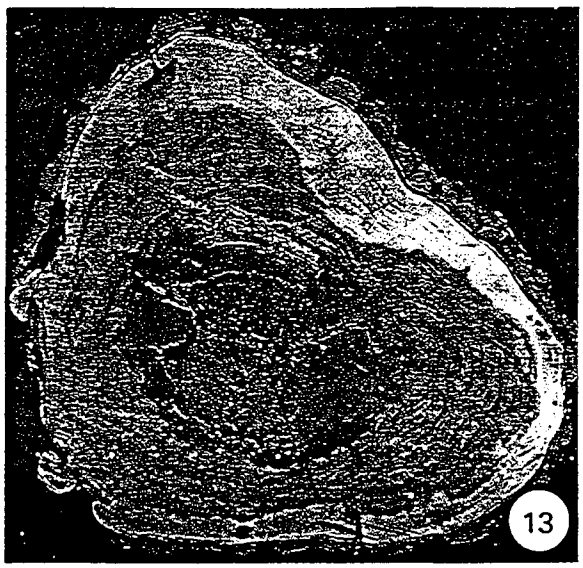


Figure 13. Brown butt rot of balsam poplar, probably caused by *Armillaria mellea*. Figure 14. Butt rot (brown rot) of aspen caused by *Armillaria mellea*. Figure 15. Black gall on aspen.

al. 1960). Decay caused by unknown organisms accounted for 38.2% (34.7% of trunk infections and 3.5% of butt infections), which is greater than decay volume due to *P. tremulae* (26.8%) (Thomas et al. 1960). More investigations are needed to determine the unknown organisms causing the decay in this species and the relative importance of decay fungi.

Many bacteria and other fungi are constantly isolated from decayed and stained tissues (Etheridge 1961; Good and Nelson 1962; Laflamme and Lortie 1973; Shigo 1967; Thomas et al. 1960). These are not considered decay-causing organisms, but they are believed to play an important role in microbial succession leading to incipient decay in aspen and poplar.

On trembling aspen, blackish galls with a rough surface are often found (Fig. 15), but the interior of the galls is sound and not associated with decay. Except for one tree with large scars and conks, about 20 trees with black galls did not have conks and did not have advanced decay[1].

AGE-DECAY RELATIONSHIPS

Aspen and balsam poplar are shade-intolerant species, hence they tend to grow in even-aged stands. These stands originate primarily by means of suckering after forest fires or logging. Regeneration of shade-intolerant aspen and balsam poplar ceases when the forest canopy is closed.

Age-decay relationships are usually tabulated in 10-20 year classes; therefore, all published age-decay relationships show decay percentages of aspen and balsam poplar that are ranked by the ages of unrelated, noncomparable aspen and balsam poplar stands. Because each stand is unique with respect to fire history, genetic origin, site quality, and species composition, there is a great variability in the data published by different researchers. Tables 2 and 3 demonstrate how aspen decay and balsam poplar decay percentages vary across Canada and the United States. A second major cause of the high variability of data published on age-decay relationships in aspen and balsam poplar relates to the lack of clear distinction between incipient and advanced decay and between stain and incipient decay. A third cause of variability is the selection of different assessment standards in the various decay and cull surveys.

Pedology Consultants (1982) analyzed 1015 trees in the O'Chiese Block of the Rocky-Clearwater Forest. Fire history in this forest district has restricted the growth of aspen and balsam poplar stands to single age classes. Eighty-five percent of their 1015 sample trees were over 45 years old, and the average age was 70 years. Their estimated decay percentage was 8.7% for aspen and 7.4% for balsam poplar and did not include unmerchantable stems. Only merchantable sawlogs with less than 50% decay and having more than 9.0 cm top diameter were analyzed. Bailey and Dobie (1977) employed similar standards of utilization, and obtained comparable results (Tables 1 and 2). Paul and Etheridge (1958) analyzed decay and cull relationships at the logging site. For aspen they show nearly five times more decay (30%) in the 51-60 year age class than Bailey and Dobie (6.2%), five times more decay in the 71-80 years age class, and three times more decay in the 91-100 years age class. For balsam poplar, Bailey and Dobie show nearly equal decay percentages throughout 140 years of age (range 0.7%), while Paul and Etheridge show a range of 21.6% through 170 years of age.

In general, decay percentages of gross volume increase with age; however, comparisons between published results obtained by different researchers across Canada and the United States are difficult. Although preselection and rejection of unmerchantable logs at the logging site give low decay incidence figures in some surveys (Bailey and Dobie 1977; Pedology Consultants 1982), the high labor costs of felling, cutting, and selecting may make the operation uneconomical.

SITE-DECAY RELATIONSHIPS

Frequently, a wide range of diameters exists in even-aged aspen stands because of variations in site quality and competition by surrounding trees. This makes it difficult to obtain correlations between tree age and diameter. When diameter limit cuts provided the stems for decay and volume analyses (Bailey and Dobie 1977; Pedology Consultants 1982), poor correlations were found between age and decay (Tables 2 and 3).

[1] Field observations at Blue Ridge, Alberta, in 1982 by Y. Hiratsuka and A.A. Loman.

Table 2. Age-decay relationships in aspen stands in Canada and the United States

Age class (years)	Percentage decay							
	Minnesota[a]	Manitoba-E. Sask.[b]	Petawawa, Ontario[c]	Upper Pic, Ontario[d]	Lesser Slave Lake, Alta.[e]	Lesser Slave Lake, Alta.[f]	Colorado[g]	Rocky-Clear-water, Alta.[h]
31-40	1.4	—	2.9	—	12.0	—	—	—
41-50	—	1.8	4.4	—	14.0	—	—	—
51-60	4.7	3.2	5.9	1.0	30.0	6.2	6.5	—
61-70	5.9	7.1	7.4	—	40.0	—	—	—
71-80	5.6	14.4	8.9	3.0	42.0	8.4	14.5	8.7
81-90	3.0	21.3	—	—	40.0	—	—	—
91-100	11.5	25.2	—	5.0	36.0	12.2	23.0	—
101-110	18.1	31.1	—	—	33.0	—	—	—
111-120	26.5	36.9	—	8.0	30.0	—	31.0	—
121-130	41.5	41.9	—	—	—	—	—	—
131-140	10.3	—	—	8.0	—	—	45.0	—
141-150	22.5	—	—	—	—	—	—	—
151-160	—	—	—	15.0	—	—	48.5	—
161-170	—	—	—	—	—	—	—	—
171-180	—	—	—	17.0	—	—	—	—

[a] Meinecke (1929).
[b] Black and Kristapovich (1954).
[c] Riley (1952).
[d] Basham (1958).
[e] Paul and Etheridge (1958).
[f] Bailey and Dobie (1977).
[g] Hinds and Wengert (1977).
[h] Pedology Consultants (1982).

Better correlations between site and decay were obtained when the factors of site quality and competition were considered. Paul and Etheridge (1958) divided the sample trees into fast- and slow-growing classes. Slow-growing aspen and poplar occur as a rule on the poor sites and very rarely make up the understory trees on the better sites. The fast-growing trees usually occur on good sites but may occasionally include some dominants from the poorer sites. The diameter classes yielding the maximum net increments in fast- and slow-growing aspen were 35 cm and 30 cm, respectively. In fast- and slow-growing balsam poplar, the diameter classes yielding the maximum net increment were 58 cm and 56 cm, respectively. It appears, however, that genetic factors significantly affect site-decay relationships in aspen.

CLONE-DECAY RELATIONSHIPS

In aspen, highly significant differences have been found between clones in percentage decay, volume of decay, gross volume, and net volume (Kemperman et al. 1978; Wall 1969, 1971). Wall (1971) reported that each clone had a unique pattern with respect to position of rot columns within the stem (Fig. 16) and the major types of rot present. Adjacent or intermingled clones apparently on the same site differed significantly

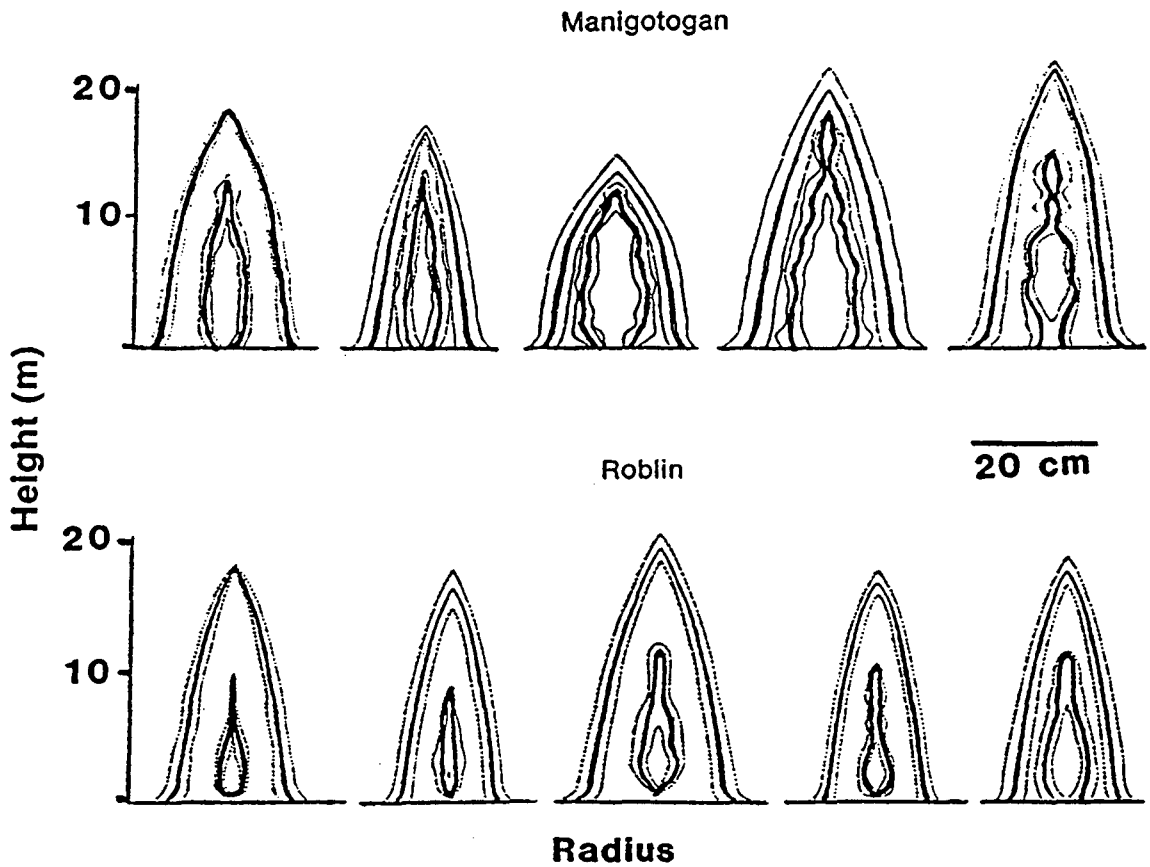


Figure 16. Representation of average tree dimensions and size, position, and shape of decay columns in some aspen clones in two Manitoba locations (Manigotogan and Roblin). Solid outer lines represent bark layer. Inner solid lines indicate decay columns (from Wall 1971).

Table 3. Age-decay relationships in balsam poplar stands in Alberta

Age class (years)	Percentage decay		
	Lesser Slave Lake		Rocky-Clearwater forests[d]
	1958[a]	1977[b,c]	
31-40	2.9	—	—
41-50	8.7	6.2	—
51-60	8.2	—	—
61-70	7.4	—	—
71-80	8.4	5.0	7.4
81-90	10.1	—	—
91-100	11.4	5.5	—
101-110	13.3	—	—
111-120	15.2	—	—
121-130	17.3	—	—
131-140	19.4	—	—
141-150	21.3	—	—
151-160	22.9	—	—
161-170	24.5	—	—
171-180	—	—	—

[a] Paul and Etheridge (1958).
[b] Bailey and Dobie (1977).
[c] Preselected logs.
[d] Pedology Consultants (1982).

in percentage decay in several instances. Components of the same clone occupying two distinguishable sites did not differ significantly in percent decay in any of the clones harvested, but considerable differences in gross volumes and decay volumes were observed between clones. In one study (Wall 1969), the incidence of *P. tremulae* among intermingled clones on the same site varied from 12% to 64%; on another site the variation ranged from 21% to 92%.

The above observations indicate that the genetic origin of aspen is more important than site quality. When aspen and poplar utilization become economically attractive, the identification of genetically healthy aspen clones will be important in regeneration programs (Steneker 1976; Steneker and Wall 1970).

No published work is available on clones of balsam poplar.

CULL ESTIMATION WITH EXTERNAL INDICATORS

Attempts have been made to find a method to predict relative incidence and extent of decay before harvesting is planned. Most of the published work uses external indicators such as sporophores (mainly conks of *P. tremulae*, Fig. 8), rotten knots, and main stem scars (Figs. 10, 17-20) (Bailey 1974; Bailey and Dobie 1977; Basham 1958; Riley and Biers 1936) to predict decay in the tree stems. Balsam poplar does not produce fruiting bodies of *P. tremulae* easily, and external indicators of decay are mainly rotten knots and large scars (Fig. 10).

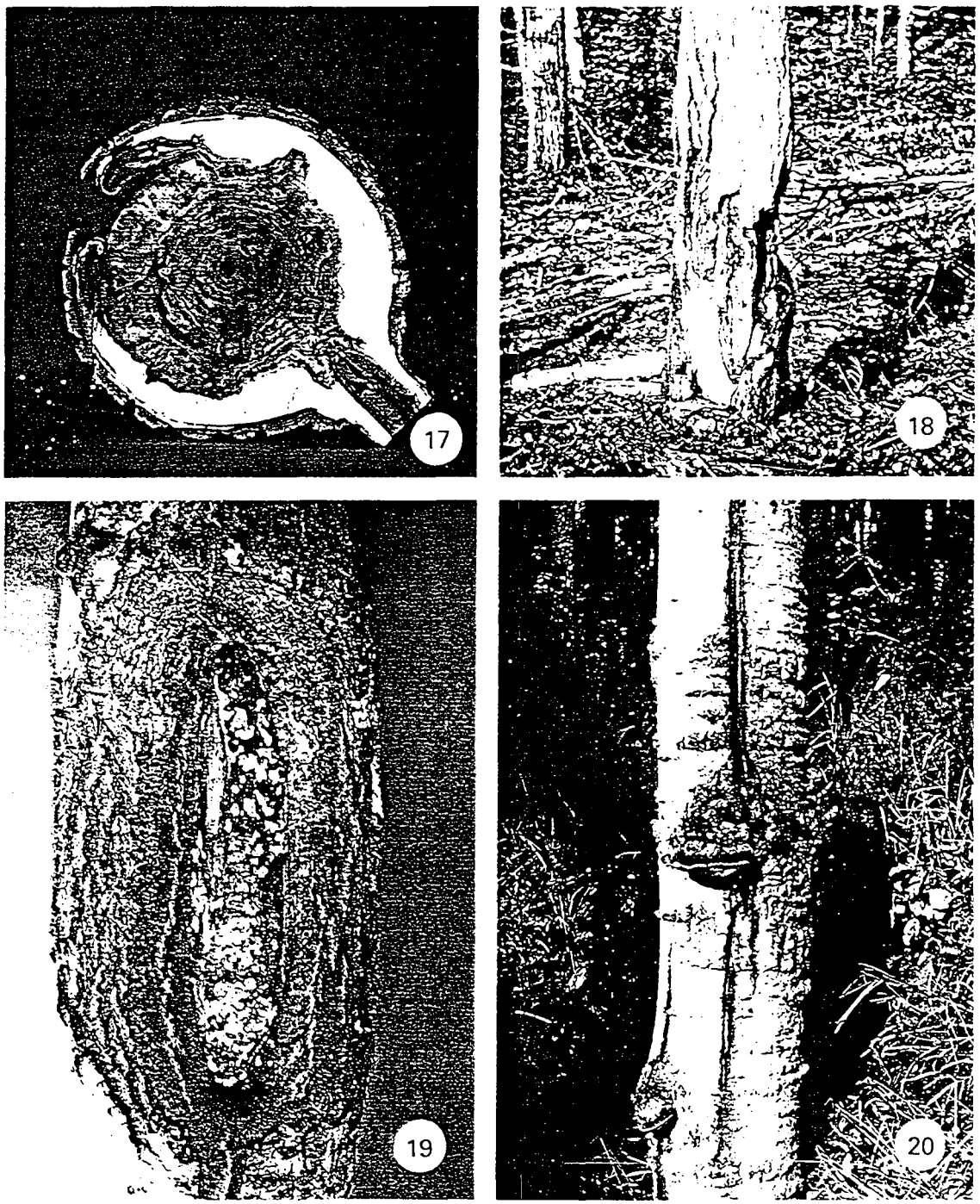


Figure 17. Decay of balsam poplar showing a scar and a branch knot as the entry points for an unknown decay organism. Figure 18. Extensive stem damage at the bottom of a mature balsam poplar. Figure 19. An old healed wound on a small diameter aspen with fruiting bodies of *Peniophora polygonia*. Figure 20. A long scar on a mature aspen with conks of *Phellinus tremulae*.

Among the indicators mentioned above, the presence of conks of *P. tremulae* was found to indicate a significant amount of advanced decay. Basham (1958) found that between 61.1% and 97.6% (average 86.2%) of trees with one or more *P. tremulae* conks had advanced decay (Table 4). At Blue Ridge, Alberta, 17 out of 18 sampled trees (94.5%) with conks and/or large scars had a significant amount of advanced decay, but only 1 of 6 trees (16.7%) without external indicators had advanced decay (Table 5). Although the number and distribution patterns of conks and other external indicators may indicate the shape and extent of decay columns, no quantitative prediction of relative amount of advanced decay can be made from examining external indicators.

Considering the strong clonal uniformity of decay patterns discussed previously, a meaningful decay prediction scheme may be established by checking several selected trees for major clones in the area concerned.

Anderson and Schipper (1978) proposed a system for predicting the amount of *P. tremulae* rot in aspen by applying a constant (1.9) to the stand basal area with *P. tremulae* conks in Minnesota.

ASPEN AND BALSAM POPLAR DECAY MANAGEMENT

Aspen and balsam poplar decay management, sound silviculture practice (Steneker 1976), utilization, and marketing are interdependent in a free market economy and cannot be discussed separately in a meaningful way. Ultimately, the aspen and balsam poplar decay management in Alberta is dependent on matching the biological characteristics of these species with 1) available utilization technology, 2) the demand for specific products on a long-term basis, and 3) the distance to markets. As long as any one of these three factors is unfavorable, aspen and poplar decay management will continue to be irrelevant in Alberta.

Several biological characteristics of aspen and balsam poplar regenerate almost exclusively from root suckers, which share a vast network of roots. Early growth after forest fires or clear-cutting is extremely rapid, giving aspen and balsam poplar (but also alder and willow species) an advantage over trees that regenerate from seeds and over competing grasses, shrubs, and herbs.

The most healthy and vigorous root suckers grow after *all* mature aspen and balsam poplar have been removed (Walters et al. 1982). Even a few residual mature trees left standing after harvesting will be dominant competitors of root suckers for available nutrients through their common root system. Walters et al. also found that partial cutting of 60-80% of basal area may maintain an aspen cover type, but remaining large trees degenerate rapidly with decay and other pathological conditions (Walters et al. 1982).

All species that regenerate vegetatively grow rapidly, but they also have very short pathological rotation ages. Harvesting at pathological rotation age means harvesting when decay rates begin to exceed growth rates, which is estimated as 40-50 years for aspen and 70-80 years for balsam poplar by Paul and Etheridge (1958), although most other decay studies indicate a higher pathological rotation age. In Alberta, harvesting at low pathological rotation age means harvesting aspen and balsam poplar of small diameters unless thinning programs are adopted (Steneker and Jarvis 1966).

Economics dictate a minimum return per hectare in terms of merchantable fiber volume. Biological constraints and the demand for forest products derived from small diameter trees determine whether the costs involved in decay management and aspen-poplar silviculture are justified. To date this has not been the case in Alberta.

If the successes from ongoing research in cellulose and hemicellulose recovery (Boone and Cyr 1981; Cyr and Schultz 1982) from stained and decayed aspen and balsam poplar can be synchronized with a demand for cellulose and hemicellulose and their derivatives, the decay management of Alberta's aspen and poplar forests will also change. For example, if up to 70% of the decayed wood contains extractable, utilizable cellulose and hemicellulose, the pathological rotation age for aspen at 40-50 years becomes irrelevant, and a different economic rotation age would be selected. If the presence of advanced decay is not the limiting factor for utilization, the continued increase in volume per unit area would be achieved at up to 100-110 years for aspen and 140-150 years for balsam poplar (Paul and Etheridge 1958).

Table 4. Relationship between visible *Phellinus tremulae* fruiting bodies and the occurrence of decay in aspen (Basham 1958)

Age class (years)	No. trees sampled	No. trees with decay	No. trees with one or more visible fruiting bodies	% of trees with fruiting bodies
41-60	131	18	11	61.1
61-80	271	52	41	78.8
81-100	553	183	150	82.0
101-120	357	179	151	84.4
121-140	165	100	86	86.0
141-160	238	170	166	97.6
161-180	39	29	25	86.2

Table 5. Relationship between decay and stain occurrence and external indicators in aspen[a] sampled at Blue Ridge, Alberta, in 1982

External indicators	No. trees	Stain and advanced decay	Stain	No decay
Conks	4	4	—	—
Conks and scars	1	1	—	—
Scars	11	10	1	—
Rotten knots	2	2	—	—
No external indicators	6	1	4	1
Total	24	18	5	1
Percentage	—	75	21	4

[a] Age range 75-80 years.

SIGNIFICANCE OF DECAY AND DISCOLORATION TO ASPEN AND BALSAM POPLAR UTILIZATION

Plywood

Plywood production demands decay-free, high-quality logs for veneer. Discoloration also downgrades the products. Besides discoloration and decay, the following problems contributed to the failure of the poplar plywood industry in Alberta (Rytz 1980a; Wells 1980).

a) The high incidence of decay resulted in enormous amounts of waste at the logging and mill sites.
b) Decay-free trees of peeler quality were scattered over vast areas in the forest, making harvesting costly.
c) Not all decay was detected in the forest, hence at the mill site the volume of waste material due to decay was enormous.
d) Poplar is difficult to peel because of ring shake, rot pockets, and tension wood.
e) Defects in veneer result in a sheet ratio that is generally below 50%. Defects are oversized knots, splits, feathered grain, and ring shake.
f) The high moisture content in poplar increases the shipping costs.
g) Higher moisture content in poplar veneer requires 15% more drying time than spruce to reduce the moisture content to 5%.
h) Dryer loss is substantial because of cracks and splits that open up in the drying process.
i) Poplar veneer requires 8-10% more glue than is required for spruce.

Dimension lumber, boards, and timbers

With respect to a previous sawmill operation in the Slave Lake area, lumber recovery from logs to merchantable material was only 15% of the total volume handled because of the presence of advanced and incipient decay. Enormous waste burners, more than double the capacity required for softwoods, were needed. Also, compared with softwoods, hardwood lumber requires 2.5 to 4.0 times as long to kiln dry (Rytz 1980a). As a result, there are the additional capital and carrying costs for extra kiln capacity. Twenty percent of poplar studs from Alberta were classified as economy grade, the lowest of four grades. This was a much greater percentage than that for softwoods.

Wafer board, flakeboard, chipboard, and oriented strand board

Clear decay-free logs are not as essential for the production of wafer board, flakeboard, and oriented strand board as they are for plywood. Decayed wood definitely affects these products, however, and could be a critical factor in the success of a mill producing them.

Pulp and paper

Excessive amounts of decayed wood processed by a pulp mill are known to affect the yield and quality of pulp and the quality of paper products (Fritz 1954; Hatton 1974; Hunt et al. 1978) MacLeod et al. (1982) reported success in designing a chip improvement process (Paprifer Chip Improvement Process) that eliminates most of the decayed wood and produces chips of the same quality as chips from sound healthy wood. If this type of process is incorporated into a pulping operation, the incidence and amount of decay would have no influence on utilization as long as sufficient sound wood was available.

Integrated wood products complex

The best way to utilize the poplar resource appears to be through the development of an integrated complex to minimize waste caused by decay, poor quality logs, and rejected finished products. The objective would be to develop as many high-value-added products as possible to offset the high logging and manufacturing costs. In the process of obtaining sound wood in the logging as well as the milling phase there is much waste, and other users must be found for the residual fiber that is generated. Such a complex could consist of a plywood plant, a wafer board mill, a sawmill, and a mill using waste (i.e., particleboard mill, chemical conversion plant). The waste can be used as an energy source for the mill. For this type of complex, the wood would be allocated as follows:

a) The highest-quality logs would be sent to the plywood mill for veneer.
b) Thirty to fifty percent of the better-quality wood would be sent to the sawmill.
c) All rejected wood from the first two processes would then be sent to reconstituted board production, supplemented by roundwood as required.
d) These three processes would generate by-products such as shavings, chips, rejects, low-quality roundwood, bark, sawdust, fines, and oversized pieces, all of which could be processed by a plant using all fibers regardless of quality.

Conversion of biomass into energy and chemicals

The advantage of considering Alberta's aspen-balsam poplar inventory as a source of biomass is that all available wood biomass would be utilized regardless of the presence and extent of decay in the wood. All cellulose materials—bole, bark, branches and twigs, foliage, and roots—can be converted to energy (Silversides 1980). Biomass harvesting for energy means being able to low-grade the forest, leaving the best trees for conventional forest products. The best harvesting system is complete clear-cutting to improve stocking, rapid growth, and natural thinning. Arguments against use of biomass for energy purposes were outlined by Kennedy (1980), who claimed that land assembly, soil depletion through short rotations, and common-sense economics make hardwood energy planning unattractive.

With the abundance of natural gas, oil, and especially coal in Alberta and British Columbia, ethanol can be manufactured more efficiently and cheaply from natural gas than from wood. It is therefore uneconomical to make wood sugar from hardwoods and then ferment it to ethanol.

Cattle feed

Steamed poplar chips have good digestibility and provide an energy component to cattle feed. The aspen-poplar inventory is a potential emergency silo for Alberta cattle (Kennedy 1980). There appears to be some problem with mycotoxins, however, which requires further study.

For this use, decay should not have much consequence, although no report has been found that discusses the food value of the decayed part of the wood.

Cultivation of edible mushrooms

Biomass of aspen and balsam poplar can be utilized to cultivate edible mushrooms such as oyster mushroom (*Pleurotus ostreatus*), the commonly cultivated mushroom (*Agaricus bisporus*), and Nameko (*Pholiota nameko*).

The presence of advanced and incipient decay should not significantly affect mushroom production if sterilized sawdust or wood chips are used. If unsterilized logs are used to cultivate mushrooms, the presence of other microorganisms in the wood, including decay fungi, will affect the growth and yield of the mushrooms.

CONCLUSIONS

1. A significant amount of early advanced decay is inherent in aspen and balsam poplar in Alberta and is caused mainly by *Phellinus tremulae*.
2. Very little information is available on decay of balsam poplar compared to that of aspen.
3. Various decay loss studies indicate that the amount of decay increases with age, although reported figures are varied.
4. Each clone seems to have its own decay pattern, which is less influenced by site or age.
5. External indicators such as conks, large stem scars, and rotten knots can be used to predict the presence of advanced decay but cannot be used to estimate the extent of decay.
6. Clone by clone predictions can be made by felling and examining several trees for the presence and amount of decay.
7. Decay appears to be less of a limiting factor when considering utilization of poplar as biomass for energy conversion and cattle feed. Intensive research and development efforts are needed to make these options viable in Alberta.

RECOMMENDATIONS

1. The identification and mapping of decay-free clones in accessible aspen and balsam poplar stands in Alberta are important and should have high priority.
2. Any aspen or balsam poplar improvement program should include the degree of clonal decay resistance as an important selection criterion.
3. Research involving the utilization of various components from trees with a significant amount of advanced decay should be encouraged. For example, research involving extractable cellulose

and hemicellulose from aspen and balsam poplar with advanced decay would be useful.

4. Since decay is only one of a multitude of factors determining the marketability of a specific product, it is recommended that decay research be integrated into studies of all other factors that contribute to the marketability of a specific product.
5. When more intensive management of aspen and balsam poplar is considered in Alberta, important biological aspects such as microbial succession leading to advanced decay, the modes and sites of decay organism infection, and the nature and heritability of clonal decay resistance should be investigated.

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CLASSIFICATION AND MEASUREMENT OF ASPEN DECAY AND STAIN IN ALBERTA

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ABSTRACT

The major types of aspen (*Populus tremuloides* Michaux) decay and stain that occur commonly in Alberta are described and illustrated. Internal stem defects are classified into five new categories on the basis of color and hardness. External indicators of decay and stain and other conspicuous stem abnormalities of aspen are described and illustrated. The use of a prototype wood hardness measuring device is outlined, and sampling and measurement guidelines are presented.

RESUME

Les principaux types de carie et de taches du peuplier faux-tremble (*Populus tremuloides* Michaux) qui se rencontrent couramment en Alberta sont décrits et montrés. Les défauts internes de la tige sont classés en cinq catégories selon la couleur et la dureté. Les signes externes de la carie et des taches ainsi que d'autres anormalités apparentes sont également décrites et montrées. L'emploi d'un prototype de dispositif de mesure de la dureté du bois est décrit, et des recommandations sur la façon d'échantillonner et de mesurer sont présentées.

CONTENTS

FIGURES

TABLES

NOTE

The exclusion of certain manufactured products does not necessarily imply disapproval nor does the mention of other products necessarily imply endorsement by Forestry Canada.

INTRODUCTION

One of the largest concerns affecting the utilization of aspen (*Populus tremuloides* Michaux) is the common presence of wood decay and stain, even in relatively young and small trees. Field recognition of types of decay and stain and standard methods to measure and record these defects are important for accurate assessment of aspen resources. Traditionally, wood defects of aspen have been classified into three categories (advanced decay, incipient decay, and stain), but that system created problems in measuring and recording defects consistently and objectively. This manual has been compiled to describe and illustrate the common types of aspen decay and stain that occur in Alberta, to present a new system of classifying defects into five categories, and to present methods of sampling and measuring these defects.

DISTRIBUTION AND UTILIZATION OF ASPEN IN ALBERTA

The volume and distribution of Alberta's aspen resource has been well documented. Approximately 35% of Alberta's growing stock is composed of hardwoods. Of this, 85% is aspen, and the balance is made up of balsam poplar (*Populus balsamifera* Linnaeus) and white birch (*Betula papyrifera* Marshall).

Aspen is widely distributed throughout North America (Fig. 1) and occurs throughout the forested area of Alberta (Fig. 2). Pure stands are more common in the northern areas.

The total volume of aspen in the province is approximately 850 million m^3. The merchantable volume is currently 831 million m^3, based on a minimum 15-cm stump diameter (outside bark) and up to a minimum 10-cm top diameter (inside bark)[1]. The distribution of merchantable volume by forest is shown in Table 1.

The annual allowable cut of aspen in Alberta is 10.4 million m^3, of which 2.9 million m^3 (27.7%) is currently allocated. The committed wood supply is evenly split between holders of forest management agreements (FMAs) and those not holding FMAs. Utilization of aspen has been well documented recently by Ondro (1989). A summary of the current and planned utilization of the aspen resource in Alberta is presented in Table 2. The current industries utilizing aspen provide approximately 1381 person-years of employment, of which 79% is primarily in the panelboard and pulp products field. An additional 3252 person-years of direct employment and 6505 person-years of indirect employment will be generated as a result of proposed development of this resource (Ondro 1989).

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[1] Timber Management Branch Statistics 89-01-31, Amendment 8, Alberta Forest Service, Edmonton, Alberta.

Table 1. Distribution of aspen merchantable volume in Alberta

Forest	Volume (million m^3)	Forest	Volume (million m^3)
Athabasca	49.22	Rocky–Clearwater	28.26
Bow–Crow	7.10	Slave Lake	142.81
Edson	37.31	Whitecourt	67.64
Footner Lake	114.41	Outside managed areas[a]	113.58
Grande Prairie	100.42		
Lac La Biche	56.51	Total volume	831.16
Peace River	113.90		

[a] Areas outside of Commercial (Green) Zone and located mainly in Agricultural (White) Zone.

Table 2. Current and future utilization of aspen in Alberta (Ondro 1989)

Product	Current utilization		Future development		Total	
	No. of mills	Wood supply (m^3/yr)	No. of mills	Wood supply (m^3/yr)	No. of mills	Wood supply (m^3/yr)
Pulp and paper	2[a]	220 632[a]	4	4 395 000	6	4 615 632
Oriented strand board	3	1 083 133			3	1 083 133
Sawn boards	117	58 155			117	58 155
Firewood	N/A[b]	41 500			N/A	41 500
Pallets	2	31 300			2	31 300
Furniture	2	430			2	430
Feed pellets	1	900			1	900
Total	127	1 436 050	4	4 395 000	131	5 831 050

[a] Estimates for two pulp mills are for trial runs of one-half year of production.
[b] N/A = not available.

Figure 1.

Distribution of aspen (*Populus tremuloides*) in North America (Harlow et al. 1979).

MAJOR CAUSES AND CATEGORIES OF DECAY AND STAIN

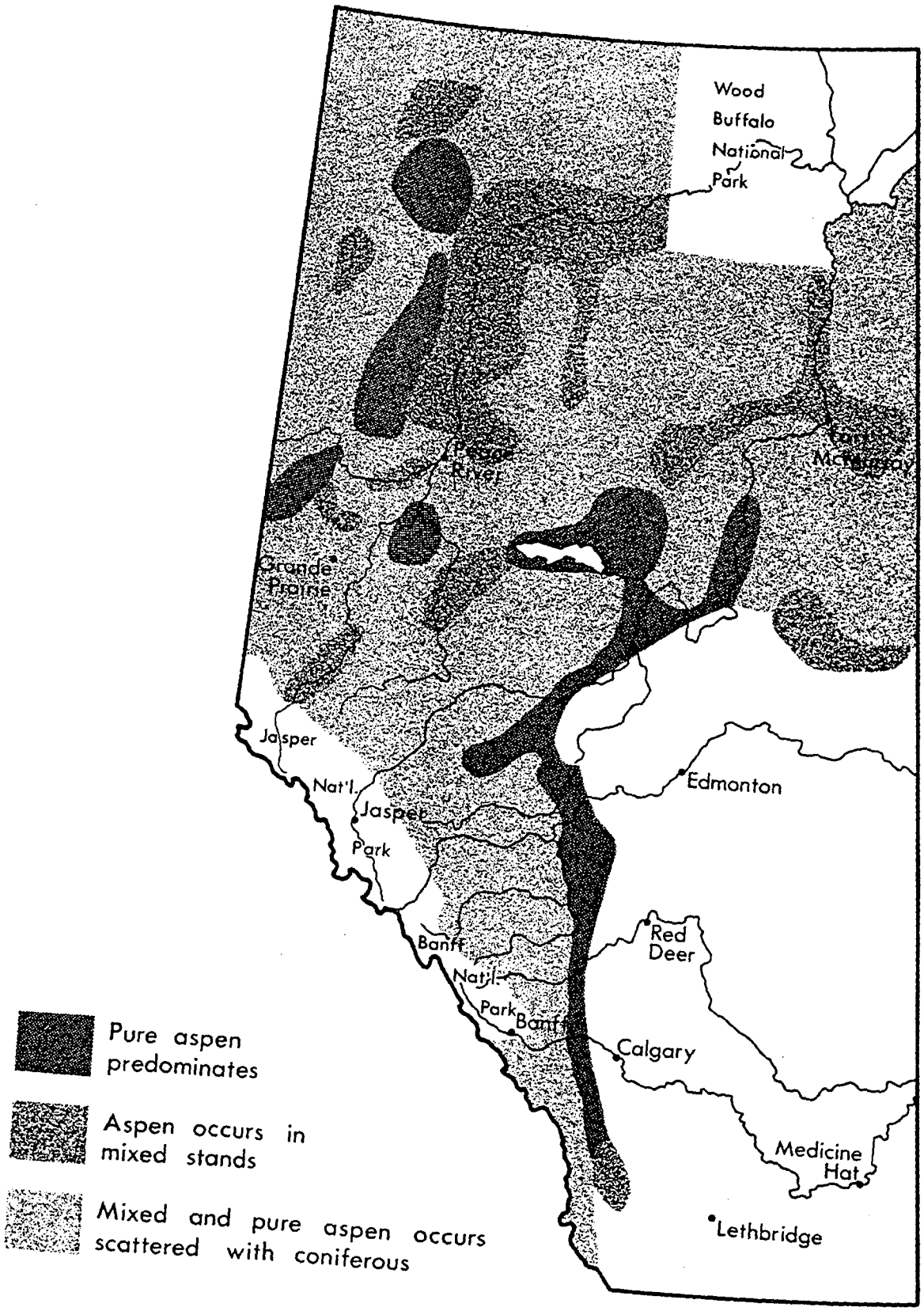
Major Causes

More than 250 species of fungi are known to cause or be associated with decay in North American aspen (Lindsey and Gilbertson 1978). Most are decay fungi of standing dead or fallen trees and are of minor importance to live aspen. Thomas et al. (1960) identified 17 species of fungi that cause the decay of standing live aspen in Alberta. Decay and stain of aspen can be divided into three major categories: trunk rot and stain; root and butt rot; and sapwood decay and stain in stored logs.

The most common and most important cause of aspen trunk rot in Alberta is ***Phellinus tremulae*** (Bondartsev) Bondartsev & Borisov (= *Phellinus igniarius* (Linnaeus: Fries) Quélet, *Fomes igniarius* (Linnaeus: Fries) J. Kickx fil. f. *tremulae* Bondartsev) (Fig. 3). Thomas et al. (1960) estimated that 38.6% of trunk decay volume is caused by this fungus. In Ontario, Basham (1960) reported that 63.2% of 1754 trees on 47 plots had trunk rot and almost 75% of the volume loss was attributed to *P. tremulae*.

The second most prevalent cause of decay in aspen is ***Peniophora polygonia*** (Persoon: Fries) Boudier & Galzin (= *Corticium polygonium* (Persoon: Fries), *Cryptochaete polygonia* (Persoon: Fries) K. Karsten) (Fig. 4). Although this fungus does not cause large columns of advanced decay as does *Phellinus tremulae*, it is found more often in decayed and discolored wood than *P. tremulae*.

The ***Armillaria*** species (*A. ostoyea* (Romagnesi) Herink or *A. sinapina* Berube and Dessureault) (Fig. 5) is the most common cause of


Wood
Buffalo
National
Park
Peace
River
Fort
McMurray
Grande
Prairie
Jasper
Nat'l.
Park
Jasper
Edmonton
Banff
Nat'l.
Park
Banff
Red
Deer
Calgary
Medicine
Hat
Lethbridge
Pure aspen predominates
Aspen occurs in mixed stands
Mixed and pure aspen occurs scattered with coniferous

aspen butt rot in Alberta. The causal agent of Armillaria root and butt rot has until recently been considered to be *Armillaria mellea* (Vahl: Fries) P. Kummer but now is considered to be caused by five or six closely related but different species. In Alberta, at least two and possibly more species are known to exist (Mallett 1985; Mallett and Hiratsuka 1988). Other common root and butt rot fungi are *Ganoderma applanatum* (Persoon) Patouillard, *Fomitopsis pinicola* (Swartz: Fries) P. Karsten (= *Fomes pinicola* (Swartz: Fries) Cooke), and *Gymnopilus spectabilis* (Fries: Fries) A.H. Smith (= *Pholiota spectabilis* (Fries: Fries) Gillet).

Another common decay organism is *Radulum casearium* (Morgan) Ryvarden (= *Hydnum casearium* Morgan, probably *Radulodon americanus* Ryvarden).

Stain or discoloration of wood is caused by various microorganisms, including fungi of various groups (yeasts, ascomyceteous fungi, and fungi imperfecti) and bacteria. One of the so-called "mineral stains" of sapwood in stored logs is likely caused by invading blue stain fungi belonging to such genera as Ceratocystis and *Verticicladiella*. Many kinds of sapwood stain are known to develop without microorganisms.

Major Categories

The widely used traditional classification system used for categorizing wood defects as advanced decay, incipient decay, and stain has created problems in judging and recording wood defects in aspen. Even among experts, there is no clear agreement on the classification of decay and stain into these categories for decay measurement purposes (Basham 1987). Inconsistencies and abnormalities in decay and stain measurements reported in various papers are likely due to the lack of standard measurement guidelines (Hiratsuka and Loman 1984).

We recommend using five categories of major wood defects for the purpose of decay and stain measurement (Table 3). A key for the identification of defect types is provided in Table 4.

Type A: Decay caused by *Phellinus tremulae*. This category is characterized by a prominent black line that surrounds and often occurs within decayed areas (Figs. 6 and 7A, B). The rot caused by this fungus is white, spongy, and soft. Most of the advanced decay category of the traditional classification was likely caused by this fungus. Type A defects caused by *P. tremulae* usually produce a long decay column, frequently continuing along most of the main stem (Fig. 6). Extensive decay of this type usually occurs more than 2 m above the ground (Basham 1987). This fungus characteristically produces distinct conks (basidiocarps) (Fig. 3B). Hinds (1963) found that the average length of the decay column above and below a conk is 370 ± 21 cm.

Figure 2.

Distribution of aspen in Alberta. (Source: Alberta Forest Service.)

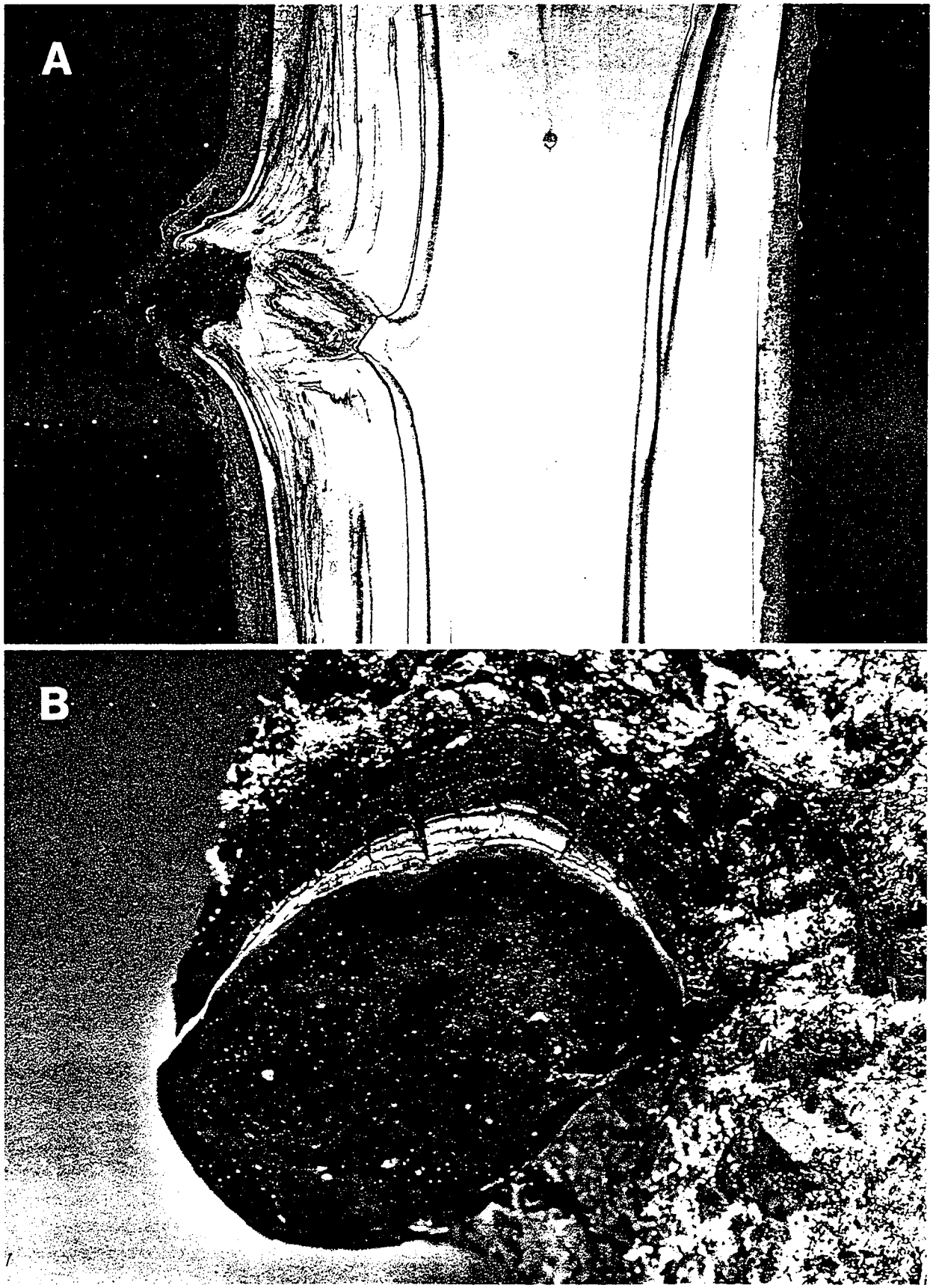

A
B

Table 3. Summary of the defect categories

Type of defect	Causes	Description of defect and external indicators	Defect distribution
A	*Phellinus tremulae*	White spongy rot bordered with black lines. Usually associated with hoof-shaped conks.	Defects usually occur along most of the main stem, less frequently in the bottom part of the trunk.
B	Mostly *Armillaria* spp.	Yellow, stringy rot often surrounded by dark brown fungal and wood material. Black shoestring-like fungal structures (rhizomorphs) present in and around the decay. May find *Armillaria* mushrooms in the late summer or autumn.	Butt rot. Decay up to 1 m above the ground.
C	Mostly *Peniophora polygonia*, occasionally *Radulum casearium*	Stained column with irregular pockets of pinkish to brownish decay. Often associated with pink scale-like fruiting bodies.	Often occurs along large portions of the main stem.
D	Various causes (fungi, bacteria, nonbiotic factors)	Stain of various causes that does not reduce wood hardness.	Variable in distribution.
E	Blue stain fungi	Grayish-black sapwood stain.	Occurs in sapwood. Initiates from cut end or through damaged bark on stored logs.

Type B: Butt and root rot primarily caused by *Armillaria* species. The yellow, stringy rot is often covered by dark brown fungal material mixed with wood (Figs. 5A, 8D, E, and 9C, D). Black, shoestring-like fungal structures (rhizomorphs) are often found within and around the decay. The Type B defect occurs only at the bottom of the tree and tapers off quickly (Fig. 9C), seldom extending more than 1 m above the ground.

Type C: Decay and stain caused by *P. polygonia* and less frequently by *Radulum casearium* and other organisms. This category of defect is characterized by a general discoloration of the wood along with pockets of decay throughout the affected column (Figs. 7C–F and 10). Decay and discoloration of wood caused by *P. polygonia* are conspicuously pink to brownish pink and can occur along large portions of the main stem. The fungus seldom causes large columns of soft structural decay, and most of the affected wood stays relatively firm. Although hardness in general may not be reduced significantly, the infected wood may be more brittle than sound wood. Fibers of the affected areas often pull out, and cut surfaces have a rough appearance, while the adjacent sound wood cuts cleanly (Fig. 7D, E).

Figure 3.

Phellinus tremulae.
A. Decay caused by *Phellinus tremulae.*
B. Conk (fruiting body) of *Phellinus tremulae.*

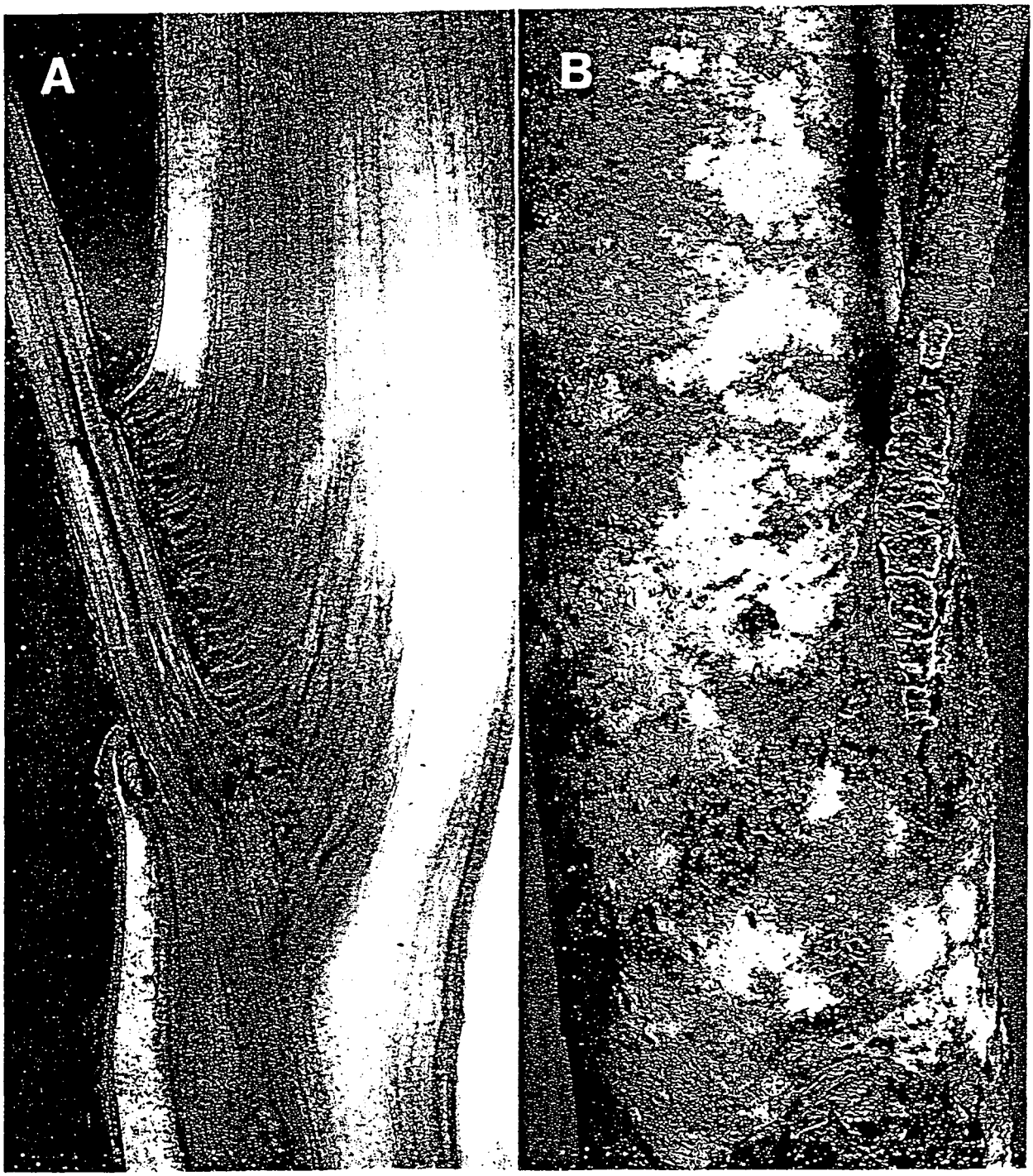

A
B

Table 4. Key to types of wood defect in aspen

Wood not discolored, no indication of defect	Sound wood
Wood discolored or with other visible indications of defect	
Heartwood defect	
Columns of structural decay	
White trunk rot bordered with black line	Type A
White or brown butt rot, seldom extending more than 1 m above the ground	Type B
Stained columns with irregular decay pockets and soft areas, mostly pink to brownish pink	Type C
Stained columns of various colors and forms without loss of hardness	Type D
Sapwood defect	
Grayish-black or brown sapwood stain	Type E

A distinct splitting of wood often occurs between affected and healthy wood areas (Fig. 10A–D), causing ring shake, which is shrinkage and separation of the annual rings. Defects caused by *P. polygonia* and *R. casearium* are difficult to separate in the field, requiring laboratory isolation to confirm their identity.

The type C defect has been the major area of confusion in the past and was likely recorded as incipient decay or stain under the traditional classification system.

Type D: All heartwood stains caused by fungi, bacteria, and nonbiotic factors, which do not reduce wood hardness as in Types A, B, and C (Fig. 8A–C). Because Type D defects are caused by various biotic and abiotic agents, they are variable in distribution and extent (Fig. 10E, F).

Type E: Grayish to blackish sapwood stains that often develop in stored logs (Fig. 8F). These stains are caused by blue stain fungi, likely belonging to such genera as *Ophiostoma, Ceratocystis,* and *Verticicladiella.* This type of stain is often included in the mineral stains category, but the name may be a misnomer as it is caused by fungi. Type E stain typically develops from log ends or damaged bark of the cut log.

Figure 4.

Peniophora polygonia. **A.** Stain and decay caused by *Peniophora polygonia.* **B.** Fruiting bodies of *Peniophora polygonia.*

There are strong indications that *Phellinus tremulae* (cause of Type A defect) and *Peniophora polygonia* (major cause of Type C defect) are mutually exclusive or antagonistic to each other (Navratil and Winship 1978). Most of the trees with Type C defect do not have Type A defect, and in trees where both types coexist there are clear demarcation lines between the areas infected by each organism. Figures 9A and B show a column of Type C defect situated in the center of the stem surrounded by Type A defect, with a very clear separation between the two. Basham (1958) considered *P. polygonia* as well as several other fungi to be preliminary fungi, which were functional in altering the host, thereby permitting the principal fungi, mainly *Phellinus tremulae* and *Ganoderma applanatum,* to become established.

10

Studies in several provinces (Basham 1958; Basham and Morawski 1964; LaFlamme and Lortie 1973; Thomas et al. 1960) showed that *Peniophora polygonia* primarily occurs in young or small diameter stems. In this study, *P. polygonia* was commonly observed in older trees as well. Further investigations are necessary to understand the ecological succession of microorganisms leading to various kinds of decay and stain.

EXTERNAL INDICATORS OF DECAY AND STAIN

Certain external characteristics can be useful in providing an indication of the presence of internal decay and stain (Fig. 11). They may signal the presence of a specific type of decay organism but do not provide a reliable estimate of defect volume.

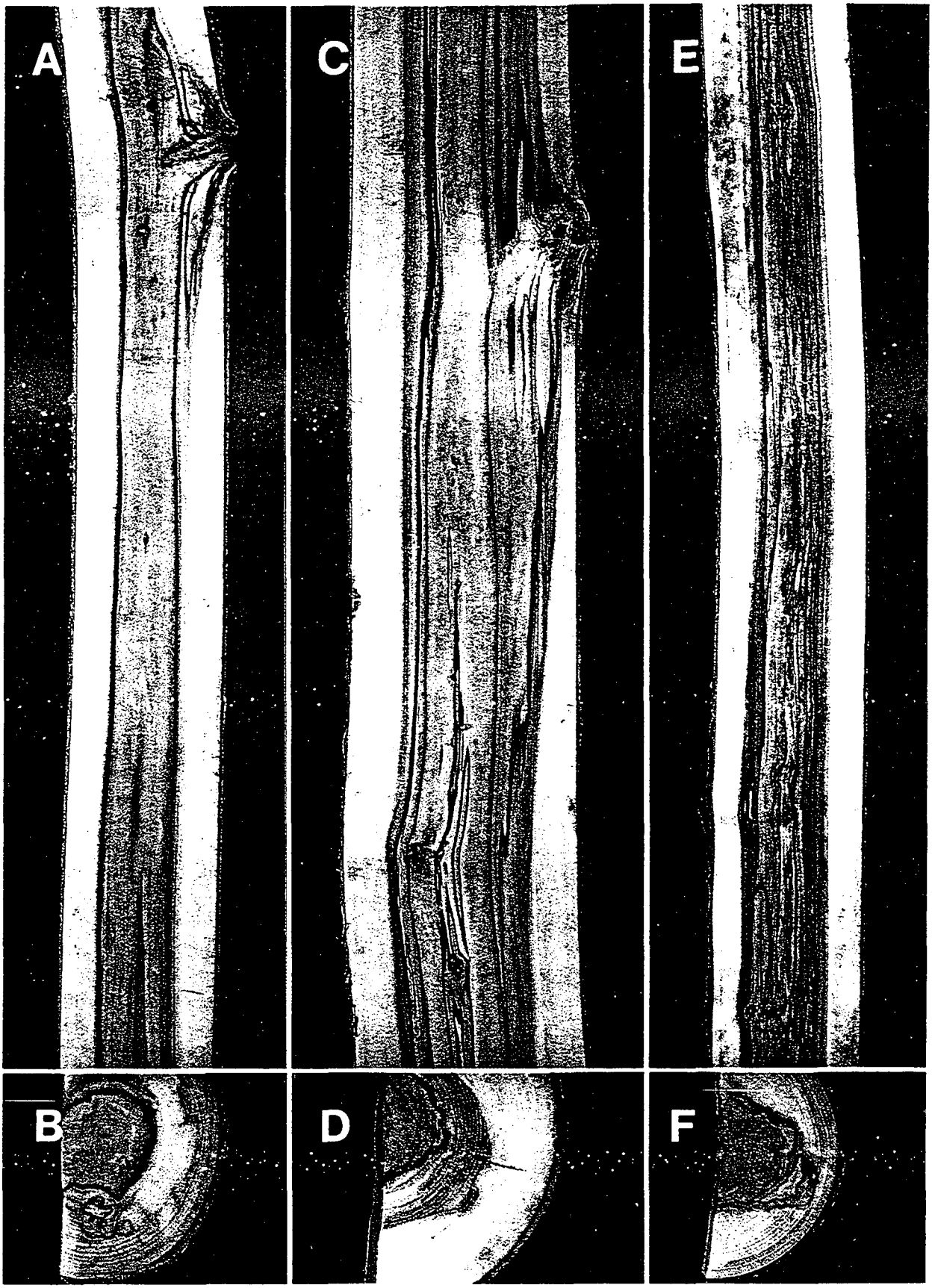
The main external indicators of decay and stain and other conspicuous stem abnormalities are

1) Perennial hoof-shaped conks of *Phellinus tremulae* (Type A defect; Figs. 3B and 11C) are produced at branch scars. The fungus is commonly called false tinder conk or false tinder fungus because of the similarity of the conks to those produced by another decay fungus, *Fomes fomentarius* (Linnaeus: Fries) J. Kickx fil., which is called tinder conk or tinder fungus. Inside fungal tissue of conks of both species have been used as tinder (punk) to start a fire. The conks can be up to 20 cm wide and 15 cm thick, but the majority are 7–15 cm wide and 5–10 cm thick. Longitudinal sections of conks are triangular and have a purplish brown lower spore-producing surface (Fig. 3B). The margin of the upper surface is smooth and pale brown but older surfaces crack and become darker, almost black. Basham (1958) found conks of this fungus on 86% of the trees with Type A defect. Our results, however, indicate a lower percentage of trees with extensive Type A defect are associated with conks.

2) Mushrooms of *Armillaria* species (Type B defects) have honey-colored to dark yellowish brown pilei (caps) and are generally 7 to 12 cm in diameter (Fig. 5B). Distinct membranous annuli (rings) on the upper stems of these mushrooms are one of the important characteristics in the identification of the fungus. The fruiting bodies (mushrooms) are produced only in the late summer or early autumn and, therefore, are not always present. Black, shoestring-like fungal structures (rhizomorphs) are always present with the Armillaria root rot infection. These rhizomorphs are often found in completely decayed wood and around the base of the infected tree. This root rot is sometimes referred to as black shoestring root rot because of the presence of this unique structure. *Armillaria* species often cause significant mortality of young conifer trees. The potential danger of this disease as a mortality-causing and growth-reducing root disease of aspen is not known; however, Stanosz and Patton (1987a, b) concluded that Armillaria root rot may significantly affect regenerated aspen stands, especially under the short rotation management used in Wisconsin.

Figure 5.

***Armillaria* sp. A.** Butt rot caused by *Armillaria* sp. **B.** Mushrooms of *Armillaria ostoyae*.


A
C
E
B
D
F

3) Fruiting bodies of *Peniophora polygonia* (Type C defect) are thin, pinkish-red scaly patches with whitish margins that often curl away from the stem surface (Figs. 4B and 11B). They are usually found on rotten branch stubs or old exposed scars. Fruiting bodies of *P. polygonia* can be difficult to detect in the field because of their small size and light coloring.

4) Basal tree damage (Fig. 11A) is caused by logging equipment, animal feeding or rubbing, and mechanical abrasion from other stems. It can provide an entry point for decay and stain organisms.

5) Rotten branch stubs (Figs. 4B and 11B) are considered the major entry points for decay and stain-causing organisms. Basham (1958) traced approximately 90% of the trunk rots to dead or broken branch stubs. Etheridge (1961), however, suggested that branch stubs may not be the main entry points for *Phellinus tremulae*, and conks appearing at the branch stubs might only be a lateral extension of heart rot caused by this fungus. We often observed *Peniophora polygonia* fruiting bodies on rotten branch stubs.

6) Stem cracks caused by frost or other factors (Fig. 11C) can also serve as the entry points for decay and stain organisms. Basham (1958) found that 84% of the trees with pronounced stem wounds had extensive heart rot (Type A defect), indicating that they were fairly reliable indicators of heart rot.

7) Conspicuous gall formations called black gall or blackish gall (Fig. 11D) have been observed frequently in Alberta (Hiratsuka and Loman 1984) but are not indicative of wood defects. Generally, trees with black galls do not have Type A defects. The cause of the black gall is not known.

8) Some other conspicuous stem symptoms such as rough bark caused by the fungus *Diplodia tumefasciens* (Shear) Zalasky (= *Macrophoma tumefasciens* Shear) (Fig. 11E) or cankers caused by *Hypoxylon mammatum* (Wahlenberg) J.H. Miller (Hypoxylon canker; Fig. 11F) are common in some stands, but they are not indicators of extensive decay or stain.

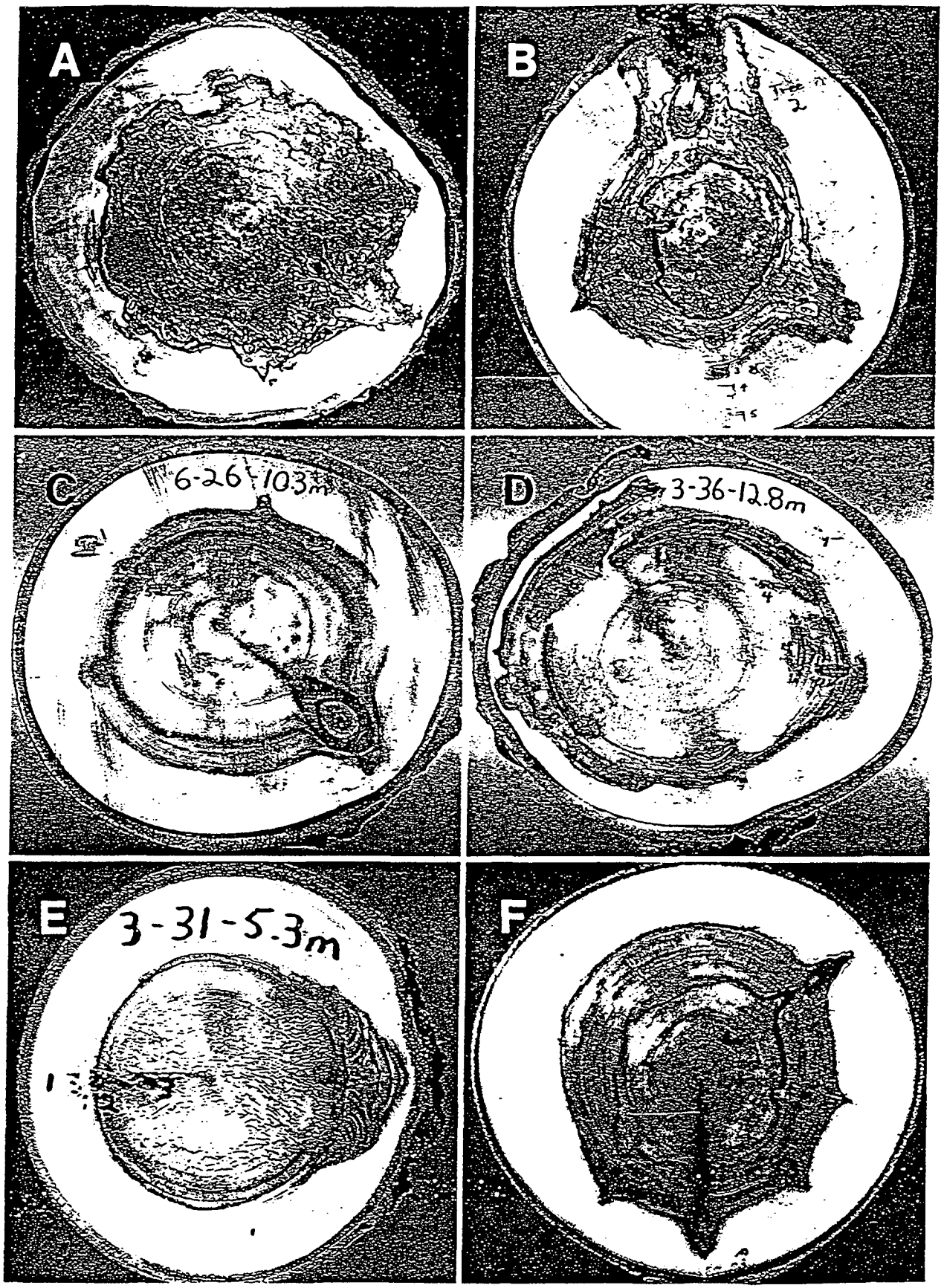
9) Stem damage caused by the yellow-bellied sapsucker (*Sphyrapicus varius varius* Linnaeus) is unique and conspicuous (Fig. 11G), consisting of regular rows of holes about 7 to 10 mm in diameter. The holes made by the yellow-bellied sapsucker often provide entry points for decay and stain organisms, but the extent of stain and decay is usually localized.

IMPACT OF MAJOR DECAY AND STAIN TYPES ON END USES

The economic consequences of the types and degree of decay and stain differ significantly according to the end use. It is important to estimate defect and yield according to the desired end use, because certain defects may affect the yield or the quality of the final product.

Figure 6.

A–F. Decay columns and cross sections of Type A defect.


A
B
C
6-26-10.3m
D
3-36-12.8m
E
3-31-5.3m
F

Bleached Chemical (Kraft) Pulping

Bleached chemical (kraft) pulping is the pulping process that is most tolerant of the presence of decay and is not affected by the presence of stain (Type D and E defects).

Results from other studies have shown that Type A and B defects result in yield losses in the form of fines (very small particles of fiber). The effect is primarily economic because the product yield, based on wood costs, is reduced. Also, the increased load on the recovery boiler caused by the fines may reduce yield in recovery-limited mills (Breck 1987).

Hunt et al. (1978) reported a significant decrease in strength of pulps produced from samples of decayed aspen (Type A and B defects). They used only decayed portions of wood for testing; however, our laboratory tests could not detect a meaningful difference in pulp strength between sound and decayed wood samples. Samples for the present study contained only an average of 20% Type A and B defects. This proportion may not have been sufficient to override inherent variability in pulp strength.

Chemithermomechanical Pulping

Chemithermomechanical pulping (CTMP) is a more sensitive pulping process than kraft pulping. As such, it is able to tolerate only low levels of stain and decay.

The effect of stain and decay on the pulping process is primarily economic, resulting in loss of yield and increased bleaching costs in order to attain required brightness levels.

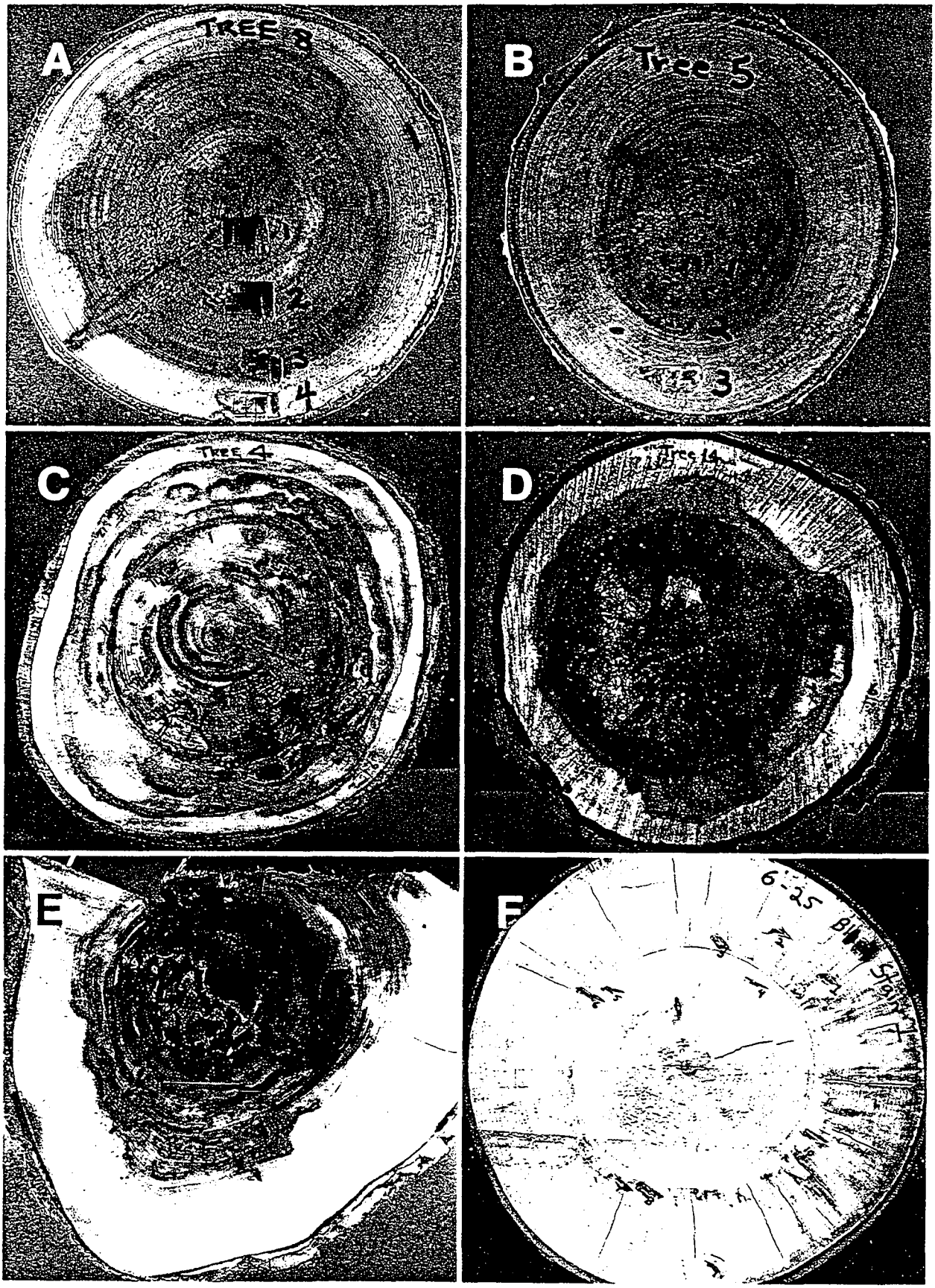
The impact of Type A and B defects is twofold. During the chipping process most of the decay is reduced to fines that are lost. Because the decayed wood must be cut, transported, and handled without a resultant increase in yield, the overall wood costs are higher. Decay that does enter the pulp results in decreased strength and requires additional bleach.

Type C and D defects present the greatest problem for the CTMP process. Bleaching costs are increased, and high brightness levels may not be attainable. If large amounts of scattered decay are present, pulp strength can be decreased.

The Type E defect characterized by sapwood discoloration (blue stain) seems to affect bleaching efficiency in the CTMP process and thus adds to the cost of bleaching. Since this defect develops mainly after logging and during storage of the wood, damage can be controlled or prevented to some extent.

Figure 7.

Stem cross sections of various defect types.
A, B. Type A defect caused by *Phellinus tremulae.*
C–F. Type C defect caused by *Peniophora polygonia.*


A
TREE 8
B
C
Tree 4
D
E
F

Oriented Strand Board

Stain (Type D and E defects) at levels of up to 20% have very little effect on oriented strand board (OSB). Technically, higher levels of stain are acceptable, but current market conditions allow for a maximum of only 20% (Breck 1987; Denny 1987).

Most decay shatters during the cutting process, producing fines that are usually lost. Fines that are incorporated into the OSB absorb more resin and affect the bulk density of the material. At high levels of decay, OSB strength can be reduced (Anderson 1987).

Lumber and Solid Wood Products

The presence of stain in more than trace amounts is not acceptable in furniture, moldings, and other specialty products because of the reduction in esthetics and strength (Breck 1987). Stain is allowed in all grades of hardwood factory lumber recognized by the National Hardwood Lumber Association. The presence of stain and decay, however, reduces the grade and subsequent value of the lumber (Petro 1987).

DEVELOPMENT OF MEASUREMENT METHODS

The following sections outline the development of a standardized method for characterizing the extent of decay. The initial identification of defects was visual, relying on the characteristic discoloration associated with stain and decay. Wood hardness was subsequently used to distinguish between defect types.

Defect Measurement in the Field

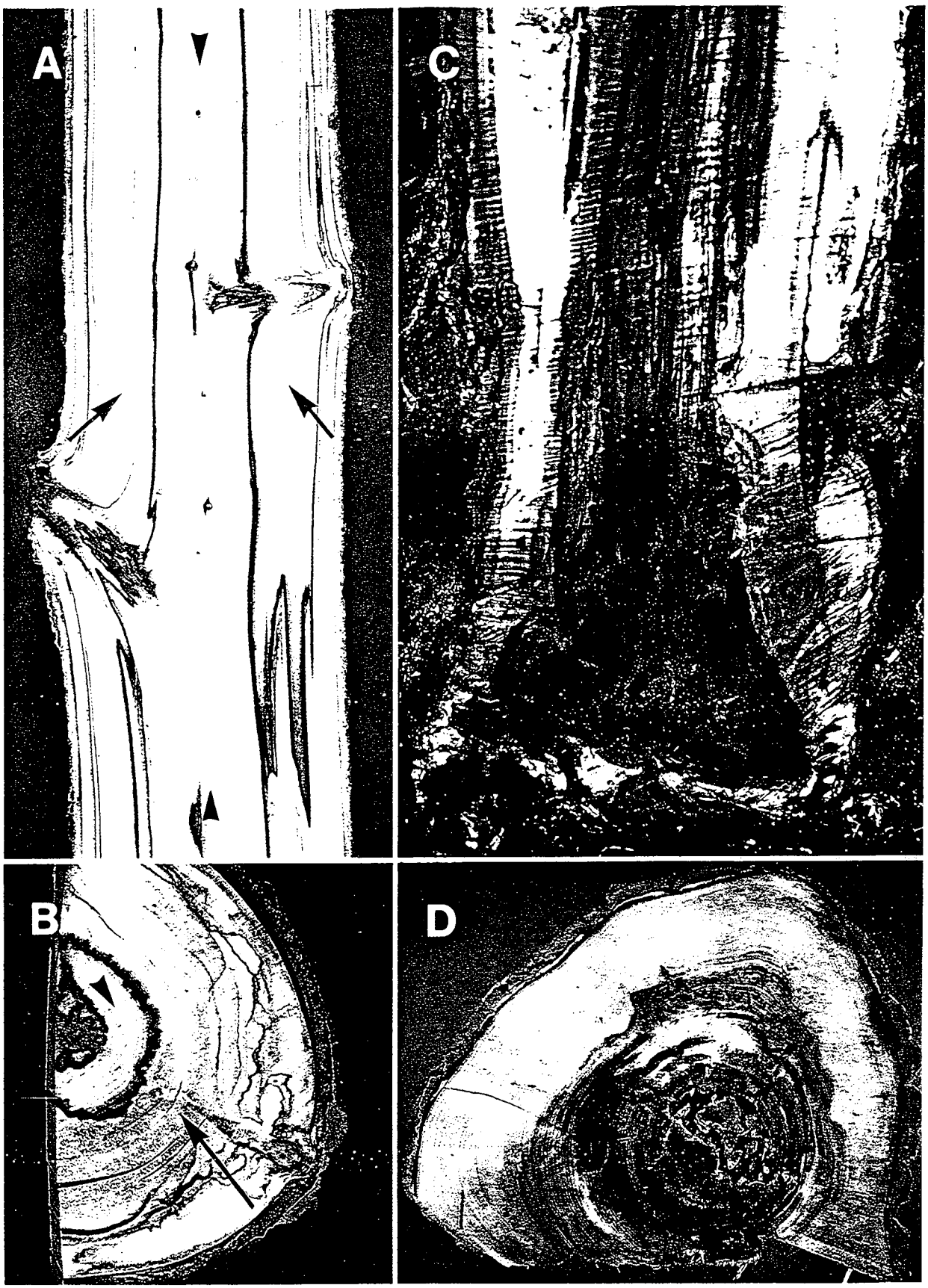
It is important to be able to easily measure the relative hardness of wood to categorize defects in the field before harvesting decisions are made. Traditional defect categorization has relied on subjective estimates of hardness utilizing knives or other sharp instruments. Several mechanical devices to measure hardness of wood and other material are commercially available, but they are expensive and inappropriate for field use.

In order to determine if defect types affect wood hardness, a prototype device called the Hoffmann-Gun or H-Gun (Fig. 12B-E) was designed specifically for measurement of aspen wood hardness[2]. The device is spring loaded, and when triggered, it releases a pin that penetrates the wood. The depth of pin penetration is related to the wood hardness, pin diameter, and spring strength. A scale measures the resistance of the wood to penetration

Figure 8.

Stem cross sections of various defect types. **A-C.** Various types of stain (Type D defect). **D, E.** Type B defect caused by *Armillaria* sp. **F.** Sapwood stain caused by blue stain fungi (Type E defect).

[2] The H-Gun was developed for this project by Walter Hoffmann of the Northern Alberta Institute of Technology, Edmonton, Alberta.


A
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by the pin. Low readings indicate little resistance, which coincides with decayed wood.

A combination of the wood's color, cut-surface texture, and trends in H-Gun readings were used to delineate the various defect types. Initially, the defects were identified using the traditional categories of advanced, incipient, and stain. Volumes of sound and defective wood were calculated based on these classes. Fortunately, the traditional classification coupled with wood hardness and defect-causing organism information could be used to categorize defects according to the system recommended in this publication.

To reduce the complexity of measurements and the confusion associated with the rather subjective incipient category of defect, we attempted to determine if a quantitative estimate of hardness could be used to distinguish decayed and sound wood. The distribution of the H-Gun readings from cross-sectional samples from each tree were plotted to determine if a logical break in readings occurred on what was classed as advanced decay, incipient decay, stain, and sound wood. The H-Gun readings for incipient decay wood did not show a clearly defined pattern. The readings ranged from 0 (minimum) to 25 (40 is maximum) and effectively covered the range of readings associated with advanced decay and stained wood.

As an arbitrary goal, we wanted to ensure that at least 75% of the readings made on incipient decay samples were categorized correctly (i.e., they were not classed as decay that would result in volume loss). The distribution of H-Gun readings on incipient samples indicated the H-Gun value of 7 was appropriate. To test this as a reliable division between advanced defects and stains, the incipient readings were divided into two groups. Samples with H-Gun values of 6 or less were put into the advanced category and those of 7 or more were combined with the stain category. Distributions of H-Gun readings indicated a high degree of accuracy (93% or greater) could be achieved if the H-gun was used to determine whether the wood should be categorized as sound or decayed.

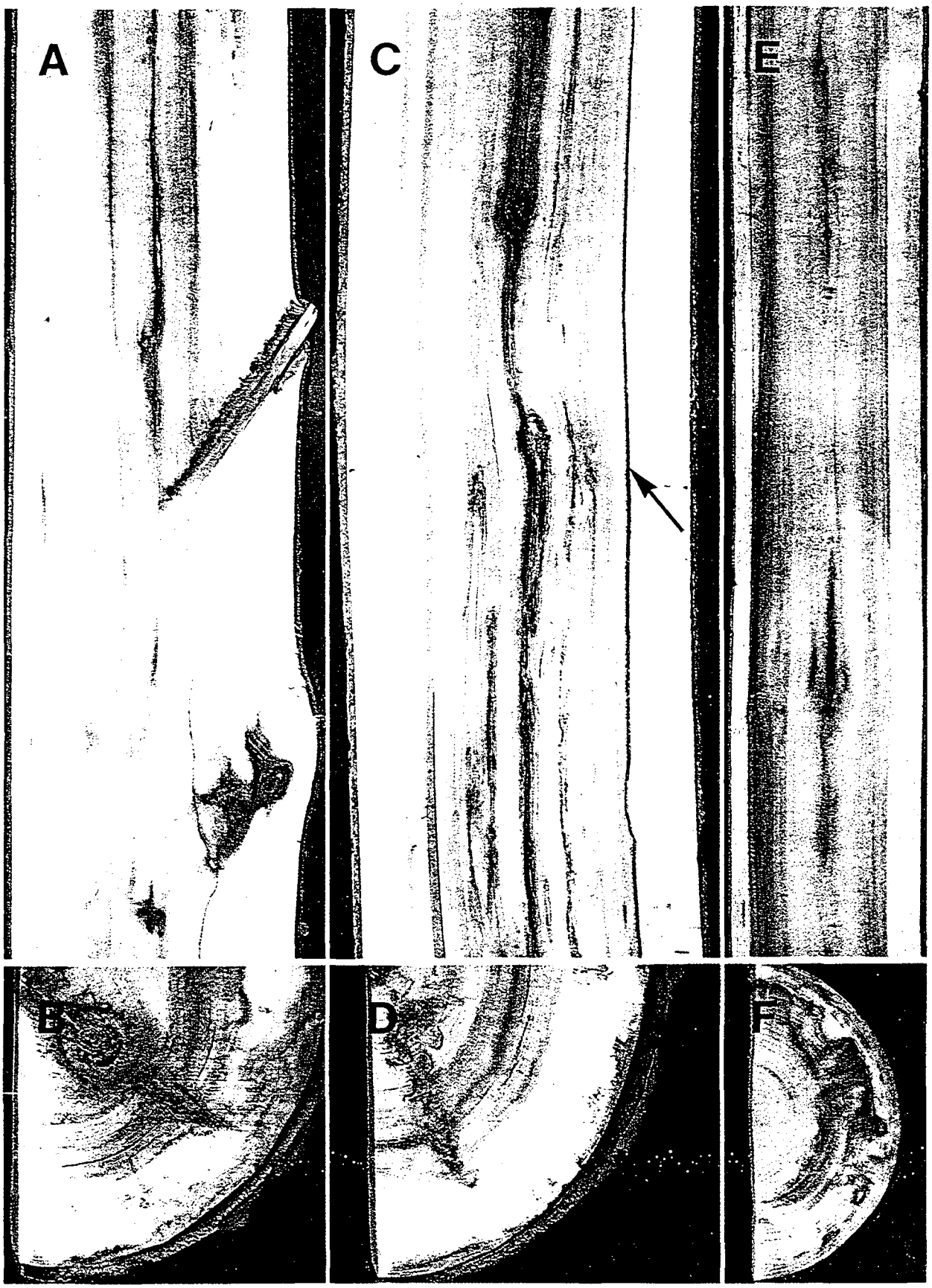
Figure 9.

Type A, B, and C defects. A. Column with Type A defect (arrows) surrounding Type C defect (arrowheads). B. Cross section of column shown in A. C. Decay column of Type B defect (*Armillaria* butt rot). D. Cross section of Type B defect.

Reconciling Field and Laboratory Measurements

Wood product testing laboratories typically provide clients with an estimate of defect based on the traditional classification of advanced and incipient decay and stain. Their assessment is based on recoverable chips and involves manually grading chips or portions of chips on the basis of color and a subjective estimate of hardness. The results are presented as a percentage of ovendry weight.

Significant discrepancies in recoverable volume derived from sound and stained wood samples were found between the field sampling and the laboratory chipping exercise. This can be attributed to three factors. The first and most important factor is likely that the criteria used in the chipping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exercise to differentiate between defect types were different from those used in the field measurements. Secondly, in the calculation of internal decay from stem analysis measurements, certain assumptions were made about the shape of the decay inside the bolt. These assumptions may not have been applicable to individual trees, but they would be reasonable over a large enough sample. Finally, field decay measurements were based on log volume, while laboratory results were determined on the basis of the ovendry weight of pulp chips.

The assumptions used in the calculation of internal defect volume from stem analysis (*Tree sectioning manual*; Alberta Forest Service 1988) were shown to be appropriate. Fifteen 2.5-m sample bolts were cut longitudinally and defect size was measured at 31-cm intervals. Comparison of these detailed volume calculations with those determined from the log end measurements revealed no significant differences ($P = 0.10$) in volumes of stained and decayed wood.

Despite the differences in defect volumes, fairly strong correlations were found between laboratory and field estimates of defect (with the exception of incipient decay). Figure 13 shows scatter plots of laboratory estimates versus field estimates of defect. Correlations between the two estimates were as follows:

Type	Correlation
Percent sound	0.52
Percent stain	0.60
Percent incipient	0.28
Percent advanced	0.79

The consistently high laboratory estimates of stain (Fig. 13B) relative to field estimates indicate that the criteria used to differentiate between defect types differed. The cause of the difference is not known but may be related to the natural variability in color or the subjective determination of wood hardness of manually graded pulp chips.

SAMPLING AND MEASUREMENT GUIDELINES

The objective of this section is to provide guidelines for future aspen defect sampling and measurement. The use of this methodology will provide some standardization for identification and measurement of wood defects in aspen and removes much of the subjectivity involved in such work. The guidelines are a first approximation; refinements and revisions to the methodology are anticipated as additional work and testing is completed.

Figure 10.

A–F. Decay and stain columns and cross sections of Type C defect.

The methods described assume the user has access to an H-Gun. The spring strength and pin thickness both influence the depth of penetration of the H-Gun pin. Because of this, the ratings used to identify decay Types A and B are dependent on the H-Gun being constructed to the same


A
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specifications as the prototype used in this investigation[3]. Any H-Gun being used for decay assessments should be calibrated against the standard to ensure the hardness criterion is appropriate.

Sampling Design

There is no standard methodology for selecting sites or trees for defect sampling. The use of external indicators can signal the presence of internal decay, but their use as a predictor of decay is limited (LeMay 1986; Maier and Darrah 1989). The exception is the presence of *Phellinus tremulae* conks, which is the only reliable indicator of internal decay that would lead to a reduction in usable volume. Indicators such as *Peniophora* fruiting bodies (Type C defect) may reveal problems with stain but likely will not result in a reduction in usable wood volume.

There is a weak relationship between aspen decay and age (Basham 1958; Black and Kristapovich 1954; LeMay 1985) and diameter (Maier and Darrah 1989). Due to the high variation in decay occurrence throughout individual stands, it is unlikely that decay estimates can be tied to stand cover type descriptions.

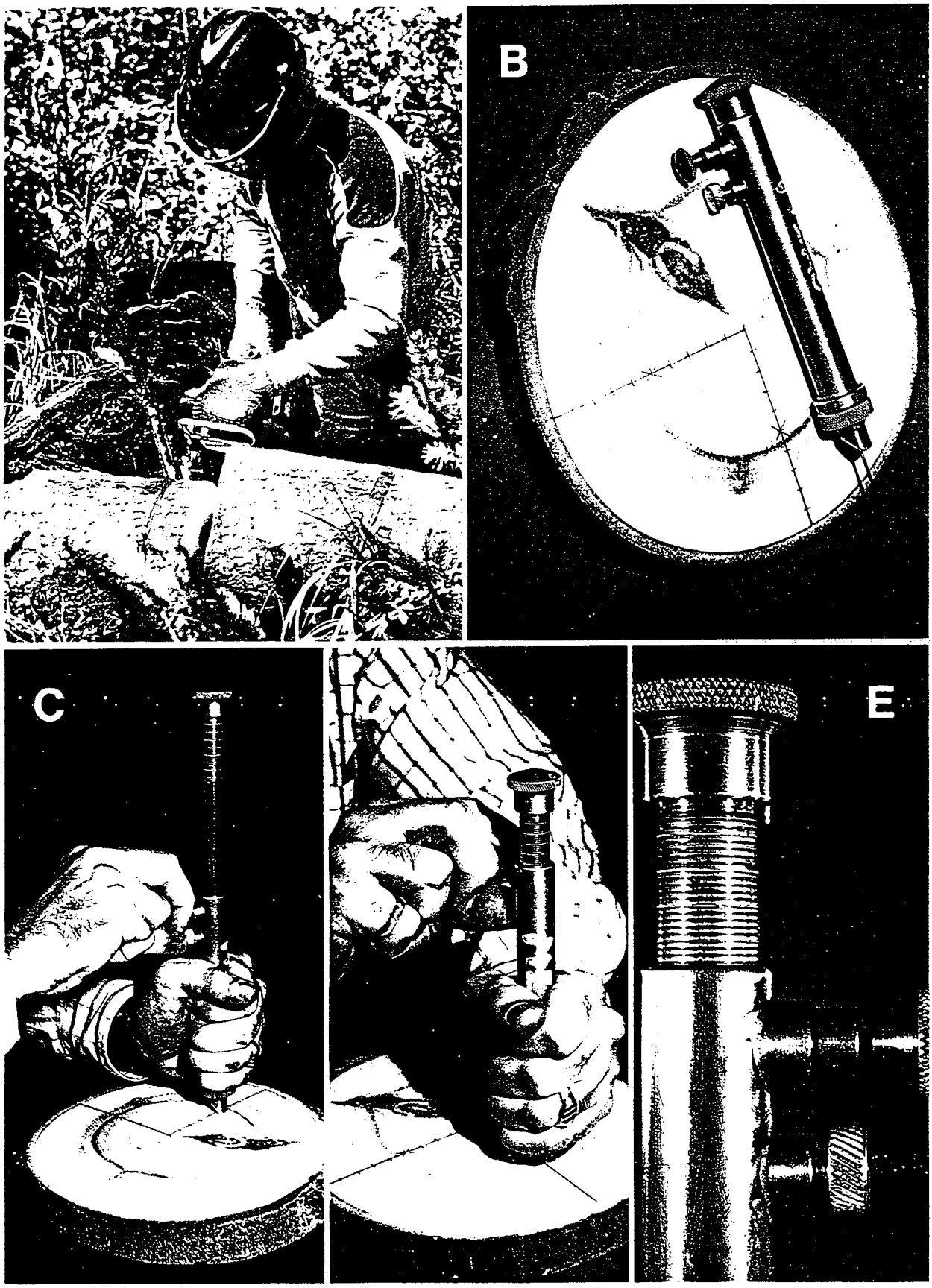
The primary consideration, then, is to sample across the range of growing conditions, stand structures, and tree sizes that are of interest. A sample of trees balanced over the geographic area of interest and over the range of tree sizes is the best approach to account for the large decay and stain variability that will be encountered. The total number of sample trees necessary to estimate decay will depend on the purpose of the survey and the size of the survey area. For an operational survey, the area of interest may be four to five townships. In this case, a minimum of 100 trees collected across the range of tree sizes will provide a reasonable estimate; however, these samples should be collected on a random basis throughout the entire survey area.

Through an efficient sample design, maximum value can be obtained from a decay survey. Individual stands representing average types should be selected. Then within each selected stand a sample plot should be established, and each tree within the plot boundary should be destructively sampled. This method has distinct advantages over sampling individual trees throughout the study area. Generally, there will be more confidence in the results, because defect estimates can be averaged to the stand level; incidences of specific defect factors (such as *Phellinus* or *Peniophora*) can be based on the stand survey; and travel costs will be reduced by sampling on a plot basis.

Figure 11.

Various external stem abnormalities of aspen. A. Basal stem damage. B. Dead branch stub with fruiting bodies of *Peniophora polygonia*. C. Stem crack with a conk of *Phellinus tremulae*. D. Black gall of unknown cause. E. Rough bark symptom caused by *Diplodia tumefascience*. F. Cankers caused by *Hypoxylon mammatum*. G. Stem damage caused by yellow-bellied sapsucker (*Sphyrapicus varius varius*).

[3] H-Gun specifications are available from the Timber Management Branch of the Alberta Forest Service. The device is not yet being produced commercially.


A
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Field Measurement Guidelines

Much of the recommended field methodology that follows is taken from the *Tree sectioning manual* (Alberta Forest Service 1988).

Felling and Sectioning

Tree attributes are tallied, with special note being taken of decay indicators, disease, stem form, and stem condition. External indicators should be assessed both prior to and after felling to ensure all indicators are detected.

Stump height and breast height are marked on the stem. The felling cuts are made below the stump mark, using standard felling techniques.

Tree length is measured and marked off in 2.5-m sections starting at the stump height. Cross-sectional samples (cookies) are taken at each mark, including at stump and breast height (Fig. 12A). All defects are noted, and their extent is determined.

To identify defect type and assist with determining the extent of defects (chasing), hardness testing should be performed in the field at the tree sectioning site.

Cookies from each tree are then bagged so that age, diameter, and defect measurements can be determined later. It was during this last phase that hardness testing took place in this study.

Determining Defect Volume

A cookie from each sectioned bolt is used in the calculation of defect volume. Because hardness testing is conducted on each cookie, these cookies must be a minimum of 4 cm thick.

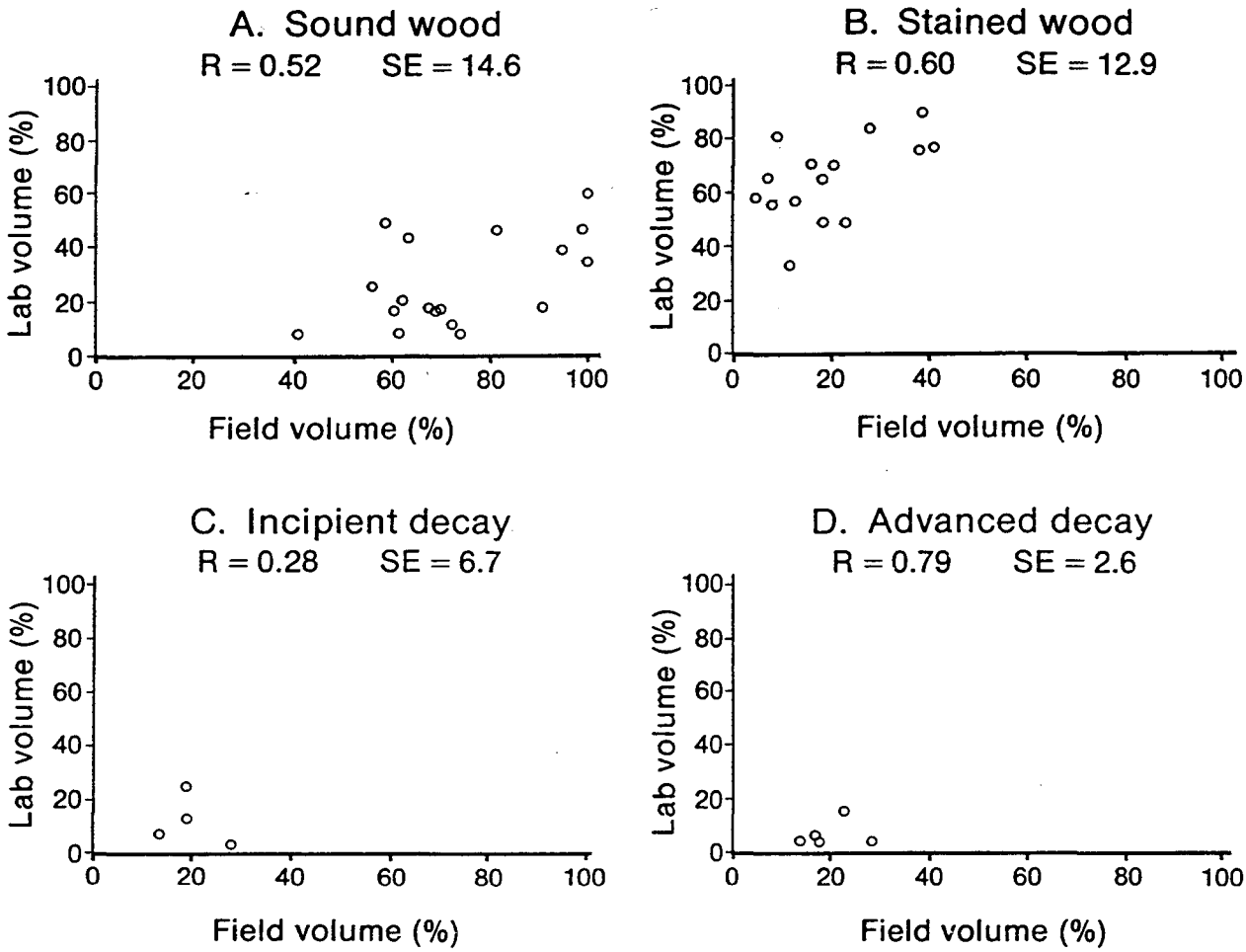
Figure 12.

Field measurement methods. A. Cutting cookies in the field. B. H-Gun and a cookie marked for testing. C. Hardness testing showing the H-Gun in the cocked position. D. Hardness testing showing the H-Gun after an application. E. The H-Gun scale after an application.

The extent of decay and stain is measured along two perpendicular radii, which are marked on the cookie (Fig. 12 B-D). These radii should be selected so that, between the two, they represent the average radius.

Hardness measurements should be taken at various intervals along the marked radii. Measurements are taken approximately every 3 cm along obviously sound or obviously decayed areas. H-Gun readings in these areas should be relatively consistent. In areas where a transition between defect types occurs, more-frequent measurements are necessary (0.5 cm apart). The objective of the H-Gun readings is to identify the transition from readings of 7 and above to those below 7.

Once the extent of each defect type has been determined for each sample cookie, the defect volumes can be calculated using the procedure outlined in the *Tree sectioning manual.*



Several options for calculating volumes exist. Each defect type (A to E) can be treated separately; alternatively, those types that cause structural decay (A and B) can be aggregated, as can the stains (D and E).

The user has several options for the treatment of Type C defect. Defects known to be caused by *Peniophora* or *Radulum casearium* but that do not contain small pockets of soft wood should usually be included with Type D defect. For chip-based products, the volume of structural defect in Type C may be insignificant; therefore, Type C defect could be included with the remaining stains (Type D and E). For solid wood products, however, scattered pockets of soft wood can make the entire wood volume unusable. In this case, Type C defect might be aggregated with Type A and B defects. In all instances, the treatment of Type C and other defects should be documented.

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Figure 13.

Comparisons of laboratory and field estimates of defect volumes.

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Appendix 3

CANADIAN JOURNAL OF PLANT PATHOLOGY 16:199–201, 1994

Perennial stem galls of aspen caused by the poplar budgall mite, *Aceria parapopuli* (Acariformes:Eriophyidae)

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Large, black perennial trunk galls occur on trembling aspen (*Populus tremuloides*) in localized areas of Alberta and British Columbia. The cause of many of these galls is unknown, but some galled trees have been previously shown to be less likely to have advanced decay caused by *Phellinus tremulae* than are surrounding nongalled trees. In a large area of trees with multiple stem galls near Dawson Creek, British Columbia, the surface morphology of galls differed from that of galls occurring in central Alberta. The Dawson Creek galls had succulent red tissue proliferations on the surface that contained poplar budgall mites (*Aceria parapopuli*), which normally occur on aspen twigs, but rarely on stems. The trees in this study appear to be of one clone and, from examination of the internal gall morphology, have likely been continuously inhabited by mites for many years. The potential of these mites to induce resistance against decay needs further study.

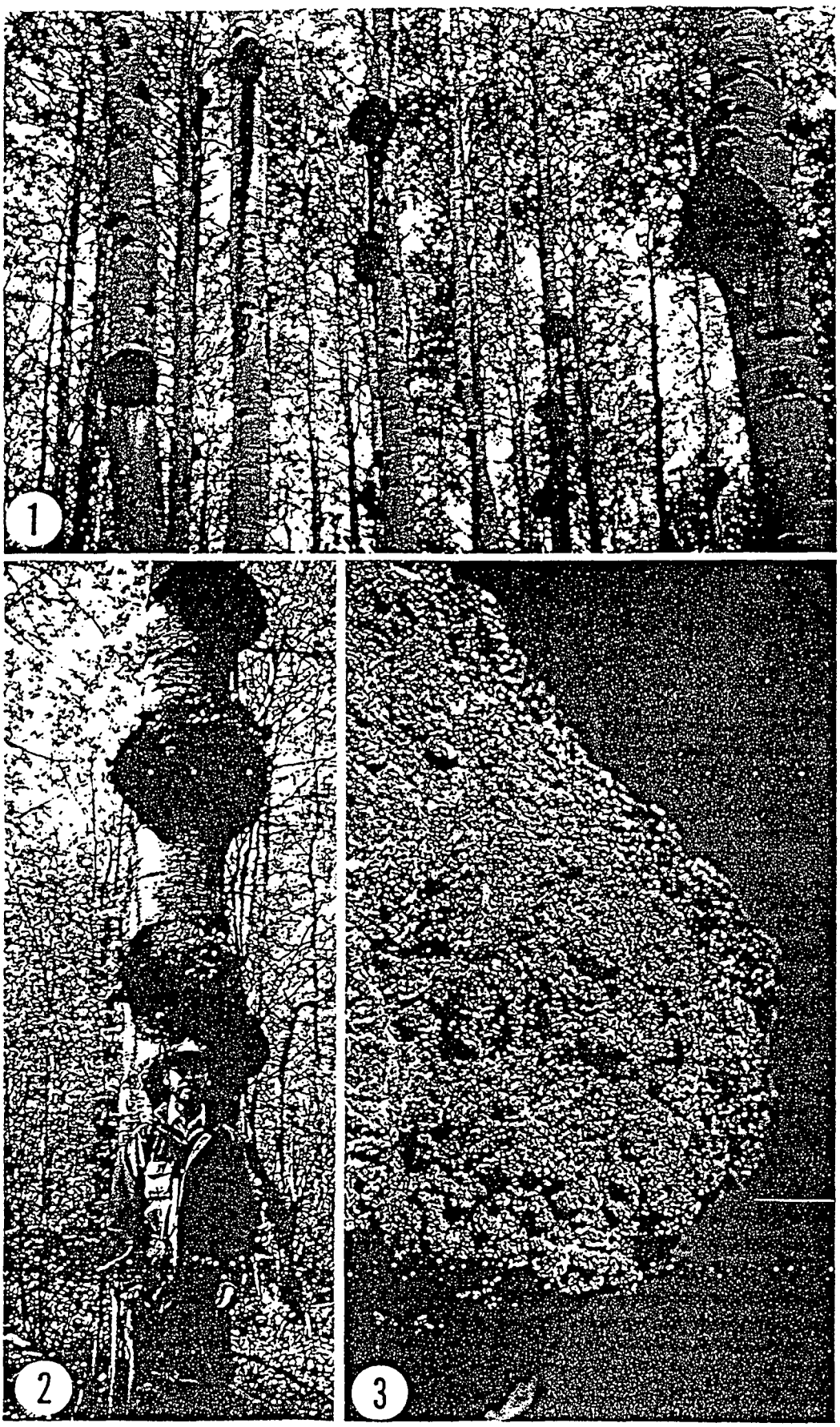
Crane, P.E., and Y. Hiratsuka. 1994. Perennial stem galls of aspen caused by the poplar budgall mite, *Aceria parapopuli* (Acariformes:Eriophyidae). Can. J. Plant Pathol. 16:199–201.

Dans certaines régions de l'Alberta et de la Colombie-Britannique, on trouve de larges galles noires permanentes sur la tige du peupliers faux-tremble (*Populus tremuloides*). On ignore la cause de la formation de bon nombre de ces galles, mais il a déjà été démontré montré que certains arbres atteints risquent moins de parvenir à un stade avancé de la pourriture causée par le *Phellinus tremulae* que des arbres avoisinants et non porteurs de galles. Près de Dawson Creek, en Colombie-Britannique, on a étudié un vaste secteur où se trouvent des arbres porteurs de nombreuses galles sur les tiges dont la morphologie de surface diffère de celle des galles observées sur des arbres du centre de l'Alberta. Les galles de Dawson Creek étaient formées de proliférations de tissu rouge succulent en surface et qui contenaient des phytotes du bourgeon du peuplier (*Aceria parapopuli*), qui sont normalement trouvés sur les brindilles du peuplier faux-tremble, mais rarement sur la tige. Il semble que les arbres de cette étude appartiennent à un même clone et, à l'examen de la morphologie interne des galles, que les phytotes s'y sont installés en permanence depuis bon nombre d'années. Le potentiel qu'ont ces phytotes d'induire une résistance contre la pourriture mérite d'être étudié davantage.

Trembling aspen (*Populus tremuloides* Michx.) is prone to many types of stem deformities. Large black stem galls occur on aspen stems in localized groups in central and northern Alberta, British Columbia (Crane 1993), and Colorado (Hinds 1985, Juzwik et al. 1978). Hinds (1985) suggested that they are insect-related; Peterson and Peterson (1992) claimed that they consist of raised callus tissue resulting from superficial wounds. Aspen with large black stem galls are less likely to have advanced decay caused by *Phellinus tremulae* (Bond.) Bond. & Boriss. (Hiratsuka & Loman 1984, Crane et al. 1994). This white-rot fungus is the most common cause of heartrot in aspen and the most severe limitation on the economic utilization of this tree species (Thomas et al. 1960, Hiratsuka & Loman 1984, Cheyne 1990). A morphological and anatomical study has shown that there are likely several causes for large black stem galls on aspen (Crane 1993). The purpose of this communication is to report the cause of one type of perennial stem gall.

An area of about 10 ha of aspen with galls on more than 80% of the trees was found near Dawson Creek, British Columbia (approx. 55°N 121°W). The galls occurred on both branches and stems, with stem galls often greater than 0.6 m across (Figs. 1, 2). These galls were unusual in having numerous red succulent, cauliflower-like proliferations on the surface (Fig. 3). These contained many eriophyid mites that were identified as the poplar budgall mite, *Aceria parapopuli* Keifer (= *Eriophyes parapopuli* Keifer). It was also determined that dried stem galls previously collected from Hinton and Whitecourt, Alberta, were also most likely caused by this mite because they bore similar proliferations of surface tissue. It was also possible to dissect out older proliferations with characteristic hairy surfaces from wood of previous years. It is therefore believed that *A. parapopuli* is the primary causal agent of these galls rather than a secondary invader.

The poplar budgall mite, described by Keifer in 1940, occurs throughout western North America, principally on the great plains and in the intermountain region (Jeppson et al. 1975), on various poplars, including aspen, cottonwoods, and hybrids. A similar mite, *Eriophyes populi* (Nal.), occurs on *Populus*



Figures 1-3. Aspen galls caused by the poplar budgall mite (*Aceria parapopuli* Keifer) near Dawson Creek, British Columbia; 1) Aspen trees with numerous stem galls; 2) Three large galls on one tree; 3) Close-up of gall showing red succulent tissue proliferations on the surface. This tissue contains living mites.

tremula L. and *P. nigra* L. in Europe (Wilson & Oldfield 1966). Galls caused by *Aceria parapopuli* are persistent and are generally found ringing the base of a developing bud or shoot (Keifer et al. 1982). Mites occupy galls for 1–4 years, or in rare cases, up to 10–15 years (Ives & Wong 1988). A 12-year-old mite-caused stem gall was reported from Miette Hot Springs, Alberta (Campbell et al. 1969). In the present study, stem galls from Hinton thought to be caused by this mite were at least 40 years old, and some of those from Dawson Creek are likely more than 80 years old. The irregular, cauliflower shape of the galls occurs because different areas have been inhabited by the mites at different times, inducing abnormal surface proliferation and localized hyperplastic growth of the sapwood. A morphological study of these galls and comparison with similar galls of unknown cause has been done (Crane 1993).

It is impossible to determine at this time whether mite-infested aspen trees have a lower decay level than do surrounding nongalled trees because of the rare occurrence of large stem galls caused by the poplar budgall mite and a low incidence of *Phellinus tremulae* at the site near Dawson Creek. The trees with multiple stem galls may represent an aspen clone that is particularly susceptible to poplar budgall mite. The potential of these mites as inducers of resistance mechanisms against decay organisms needs to be examined.

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Black stem galls on aspen and their relationship to decay by *Phellinus tremulae*[1]

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Efficient use of trembling aspen (*Populus tremuloides* Michx.) is often limited by the presence of extensive decay and stain. Aspen trees with black stem galls of unknown cause reportedly have less advanced decay caused by the fungus *Phellinus tremulae* (Bond.) Bond. & Boriss. than do adjacent trees without galls. Nine field sites having large numbers of galled aspen were surveyed for the presence of *P. tremulae* conks. Logistic regression showed that the odds of a gall tree having at least one conk was 44% of that for trees without galls, and that the occurrence of conks varied among sites and increased with increasing tree diameter. Inclusion of black galls in models used to predict decay might result in greater accuracy on sites where these galls are prevalent. Black galls may also be potential sources of biological control agents against decay-causing organisms in aspen.

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L'utilisation du peuplier faux-tremble (*Populus tremuloides* Michx.) est souvent limitée par la présence d'importantes caries et colorations du bois. Il a été observé que les arbres portant des tumeurs noires de cause inconnue sur le tronc étaient moins affectés par la carie causée par le champignon *Phellinus tremulae* (Bond.) Bond. & Boriss. que les arbres adjacents ne portant pas de telles tumeurs. Les carpophores de *P. tremulae* ont été recherchés à neuf endroits où de nombreux peupliers faux-trembles porteurs de tumeurs noires étaient présents. D'après une analyse par régression logistique, la probabilité pour un arbre porteur d'une tumeur d'avoir au moins un carpophore n'était que 44% par rapport à un arbre sans tumeur; de plus, la probabilité de présence d'un carpophore variait d'un endroit à l'autre et elle augmentait en fonction du diamètre des arbres. La prise en compte de la présence de tumeurs noires dans les modèles utilisés pour la prévision des caries pourrait permettre d'améliorer les prévisions aux endroits où ces tumeurs sont présentes. Les tumeurs noires pourraient également constituer des sources potentielles d'agents de lutte biologique contre les organismes causant la carie du bois des peupliers faux-trembles.

Introduction

In Alberta, poplar (mostly trembling aspen, *Populus tremuloides* Michx.) accounted for only 2.4% of the total timber harvest in 1980, but this increased to 18% by 1992 (Alberta Energy and Natural Resources 1980–1981, 1981–1982, 1982–1983, 1983–1984, 1984–1985; Alberta Forestry, Lands and Wildlife 1989–1990, 1991–1992). In 1990, 28% of the annual allowable cut of aspen in Alberta was allocated; 3 years later this had risen to 63% (Hiratsuka et al. 1990; Alberta Forest Service, personal communication). Efficient utilization of aspen, however, is severely limited by its lack of resistance to wood decay and staining organisms. Heartwood decay, especially by the white-rotting fungus *Phellinus tremulae* (Bond.) Bond. & Boriss. (=*Fomes igniarius* (L.:Fr.) Kickx), is the most important cause of fiber loss in living aspen (Thomas et al. 1960; Hiratsuka and Loman 1984; Cheyne 1990).

There is evidence that aspen trees having black stem galls have less decay than adjacent trees without galls (Hiratsuka and Loman 1984; Hiratsuka et al. 1990). They occur sporadically on trembling aspen in many places in Alberta and northeastern British Columbia and have also been reported in Colorado (Juzwik et al. 1978; Hinds 1985). They have not been studied extensively, having been considered of minor importance except where tree appearance is important (Ostry et al. 1989). It was recently shown that large stem galls on aspen likely have several causes. Some are induced by the poplar budgall mite (*Aceria parapopuli* Keifer) and are characterized by mite-containing tissue proliferations on the surface (Crane and Hiratsuka 1994). Other galls are generally globose with a rough, black, fissured surface (Fig. 1), and the cause(s) is unknown (Crane 1993). The present study concerns the relationship between black galls of unknown cause and the presence of decay by *P. tremulae*.

New research approaches and opportunities for reducing decay losses might result if it could be shown that the presence of galls decreases the likelihood of decay development. This is especially significant because currently there are no methods for reducing decay incidence other than attempting to minimize wounding or harvesting stands at a young age (Basham 1991). Decays cause a large economic loss, and even a small reduction in decay frequency would represent a large economic gain. Even in the absence of a causal association between galls and decay, a strong statistical

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association might be valuable in improving methods for decay prediction. Given the importance of reliable estimates of merchantable volume, any methods for improving accuracy would be valuable.

A field survey was undertaken to test the hypothesis that trees with black galls are less likely to be decayed by *P. tremulae* than are adjacent nongall trees.

Methods and materials

Nine sites in central Alberta with a high concentration of black galls were surveyed: three at Elk Island National Park (Moss Lake and Lakeview (LV1 and LV2), four near Blue Ridge (BR1–BR4), one near Edson, and one near Drayton Valley (DV). A single plot was established at each site to include all aspen trees with black galls and any interspersed or nearby trees without galls. The plots varied in size (up to about 1 ha), and boundaries such as fences or swamps were often used to delimit them. *Populus tremuloides* was the dominant tree on all sites, although most included some white spruce (*Picea glauca* (Moench) Voss), balsam poplar (*Populus balsamifera* L.), and occasionally willow (*Salix* sp.) and white birch (*Betula papyrifera* Marsh.).

The outside diameter of the stem, 1.3 m above ground, was measured for each aspen tree. No attempt was made to determine tree age because of the difficulty of counting growth rings in aspen, especially if they are decayed; furthermore, decay volume may be more accurately predicted by diameter than by age (Maier and Darrah 1989). Conks of *P. tremulae* are considered the most reliable indicator of white spongy rot of aspen (Basham 1958; Hiratsuka et al. 1990); thus, decay was evaluated solely on their presence. At least two observers agreed on the numbers of conks and galls per tree. To ensure more accurate observation of tree tops, binoculars were used.

Logistic regression (SAS Institute 1989; Hosmer and Lemeshow 1989) initially was used to model the likelihood of a tree having at least one conk as a function of the following: the presence or absence of at least one gall, tree diameter, site, and all two- and three-way interactions of these factors. Subsequently, nonsignificant effects were eliminated from the model to arrive at the most parsimonious model that adequately fit the data. Model goodness of fit was assessed by ranking the observations in order of the predicted probabilities of conk occurrence, creating 13 groups of 40–42 observations each; calculating Hosmer and Lemeshow's (1989) goodness-of-fit C statistic; and determining the attained level of significance based on a χ^2 distribution with 11 df.

Results and discussion

A total of 529 trees were assessed in the nine plots. There were 1 to 15 galls per tree, with 36, 47, and 17% of the galls present in the bottom, middle, and top thirds of trees, respectively. Figure 2 summarizes the data for all plots, regardless of diameter. The final model for predicting the odds of a tree having at least one conk was

$$C = -1.9774 - 0.8107(\text{gall}) + \Sigma\, y_i(\text{site}_i) + 0.4062(\text{diam.})$$

where

C = ln(odds of a tree having at least one conk)

gall is an indicator variable that equals 1 if a tree has a gall and 0 otherwise

y_i is the effect of the ith site on C

site_i is an indicator variable that equals 1 for the ith site and 0 otherwise, with the ninth site arbitrarily set to 0 and used as the reference site

diam. is stem diameter (dm) 1.3 m above ground

Neither the three-way interactions nor any of the two-way interactions were significant, and using number of galls instead of the presence or absence of galls did not improve the model fit. The calculated goodness-of-fit statistic was 8.01, corresponding to a P value of 0.71, thus indicating a good fit between observed and expected values.

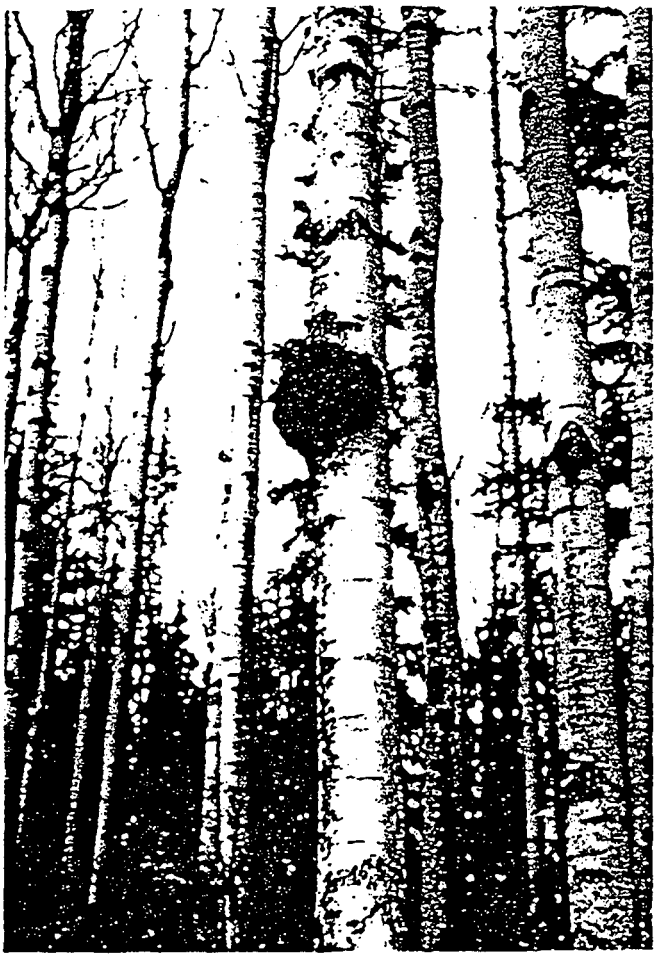

FIG. 1. A black gall on an aspen tree near Blue Ridge, Alberta.

Trees with galls were less likely to have conks than trees without galls (Figs. 2, 3). The coefficient of −0.8107 for the gall effect indicates that the odds of a gall tree having at least one conk was only $e^{-0.8107}$, or 0.44 (44%) that of a tree that was free of galls. The 95% confidence interval for this odds ratio was 28.6 to 69.1%. The absence of a significant site by gall effect indicates that the association between the presence of galls and the absence of conks was relatively consistent across sites, even though there were two sites in which conks were more common on gall than nongall trees. Given the reduced occurrence of conk formation on gall trees, it may be worthwhile to use gall occurrence as an independent variable in models for predicting stem decay.

The likelihood of conk formation varied with site (Fig. 2). The Moss Lake site had the highest occurrence of trees with conks; the odds of a conk being present were 4.1 times as great as on the reference site. At the opposite extreme, the odds of a conk being present on a tree at the BR1 site were only 0.75 times as great as on the reference site. This location effect may be related to the difference among aspen clones in decay susceptibility. It has been shown that there is a highly significant difference in aspen clones in

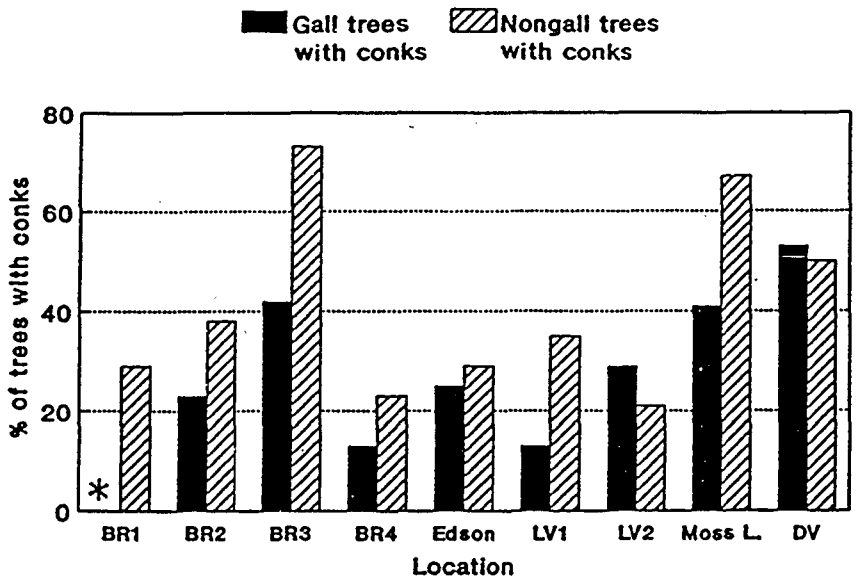

FIG. 2. For each site, the percentage of gall and nongall trees with at least one *Phellinus tremulae* conk. *, at this site, the gall trees were completely free of conks. BR, Blue Ridge; LV, Lakeview Trail, Elk Island National Park; DV, Drayton Valley.

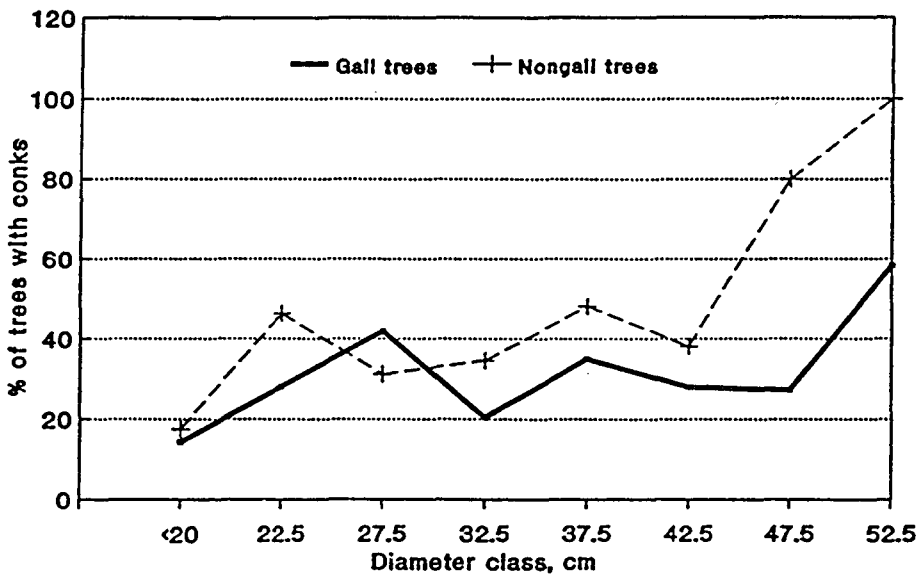

FIG. 3. Percentage, by diameter class, of gall and nongall trees with at least one *Phellinus tremulae* conk.

percentage and volume of decay; this variation is more important than site, based on percent decay in intermingled clones on the same site (Hiratsuka and Loman 1984; Wall 1971). Although it was not proven that all trees within each site were from the same clone, it is likely that this was the case for most of them because of similar bark characteristics.

The likelihood of trees having conks increased with stem diameter (Fig. 3), as would be expected, given the previously demonstrated positive association between diameter and stem decay (Maier and Darrah 1989). The regression coefficient of 0.4062 indicates that each 1 dm increase in stem diameter increases the odds ratio by a factor of $e^{0.4062}$, or 1.5. In seven of the eight diameter classes, gall trees were less likely to have conks than nongall trees. This consistency is reflected in the absence of a gall by diameter interaction and in the near parallelism of the plots of conks versus diameter for the gall and nongall trees (Fig. 3).

There are several possible reasons why trees with black galls have a lower decay incidence than adjacent trees. First, gall presence might be directly related to decay reduction if the causal organism of galls produces metabolites that protect the tree from infection or colonization by decay organisms. Three new species of fungi unique to black galls and related cankers have recently been isolated and may provide material to investigate this possibility. These are *Hyphozyma lignicola* (Hutchison et al. 1993), a new species of *Phoma* (Hutchison et al. 1994), and a black-yeast-like hyphomycete (unpublished). The *Phoma* sp. is strongly antagonistic to both *P. tremulae* and blue-staining *Ophiostoma* species. Alternatively, compounds produced by the tree in response to organisms present in black galls may enhance decay resistance. The development of persistent systemic biochemical changes in response to localized injury or infection by a large number of different agents has been

documented, particularly for herbaceous plants (Dean and Kuć 1987). Inducible systemic resistance has been demonstrated in poplars by Bradshaw et al. (1991), Davis et al. (1991), and Parsons et al. (1989), who studied the effects of mechanically wounding lower leaves in poplar to simulate herbivore chewing. They demonstrated a strong systemic response in the form of chitinase accumulation in upper leaves. Poplars have at least three different chitinase genes that are systemically wound inducible (Davis et al. 1991).

Second, decay presence may suppress gall formation. For example, *P. tremulae* produces several sesquiterpenes when grown in liquid culture (Ayer and Cruz 1993) and is antagonistic to another common aspen decay fungus, *Peniophora polygonia* (Pers.:Fr.) Boud. (Hiratsuka et al. 1990; Chakravarty and Hiratsuka 1992; Trifonov et al. 1992). Similar inhibition of the gall-causing agent is possible. Finally, a third factor may be responsible for simultaneously reducing conks and increasing galls; for example, trees genetically prone to black galls may also be genetically resistant to decay.

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Appendix 4

Benzoic acid, salicylic acid, and black galls on aspen

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ABSTRACT

Aspen (*Populus tremuloides*) bearing certain types of black galls have a lower incidence of *Phellinus tremulae* heartwood rot than do non-gall trees. Extraction of finely ground black gall tissue with ethyl acetate and separation of the acidic components of the extract led to the isolation of benzoic acid, *trans*-cinnamic acid, *p*-hydroxybenzoic acid, *p*- hydroxycinnamic acid, naringenin, 7'-methyl-hydroxynaringen, aromadendrin, and taxifolin. Bioassays revealed that among these compounds, only benzoic acid showed significant activity against *P. tremulae*. An analytical procedure was developed to measure the concentration of benzoic acid in various types of aspen tissue. Tissue from the black galls showed a high concentration of benzoic acid, and tissue from gall-bearing trees contained significantly more benzoic acid than healthy non-galled trees. However, the amount of benzoic acid present in the gall-bearing trees may not be sufficient to prevent *Phellinus* decay. It is suggested that perhaps benzoic acid serves as a precursor of salicylic acid, a signal molecule in systemic acquired resistance (SAR) of plants.

INTRODUCTION

Aspen (*Populus tremuloides* Michx.) is the most widely distributed tree species in North America (Peterson and Peterson 1992). In Canada, aspen represents more than one half of the net merchantable hardwood timber and 11% of the entire timber resource (Hunt *et al.*1978). The commercial utilization of aspen has increased dramatically in the last few years. The increasing scarcity of economically accessible softwood and the introduction of new technologies such as the chlorine-free chemothermomechanical pulping (CTMP) process have been important factors in the increased use of this hitherto neglected resource (Ondro 1989; Cheyne 1990; Peterson and Peterson 1992).

A serious limitation on the utilization of aspen is that it is very susceptible to decay and stain caused by fungi (Hiratsuka *et al.*1990). Heartwood decay, caused by *Phellinus tremulae*, is often present in a high percentage of trees in a mature stand (Hiratsuka *et al.*1990). Stored logs of aspen are very susceptible to blue stain fungi (Hiratsuka 1987) and recently we have reported on potential biological control methods for protection against these fungi (Hiratsuka *et al.*1994; Chakravarty and Hiratsuka 1994; Ayer and Jimenez 1994).

Aspen with certain types of black galls occurring as stem deformities appear to have a lower incidence of *P. tremulae* than do adjacent non-gall trees (Crane 1993; Crane *et al.*1994). The cause of these galls is unknown and they are different from those caused by poplar budgall mites (*Aceria parapopuli* Keifer) (Crane and Hiratsuka 1994). It is possible that the black galls produce compounds that protect the tree from infection, or that the tree produces phytoalexins in response to the galls and these enhance decay resistance. To investigate these possibilities, we have extracted, with various solvents, the tissue of these black galls and of aspen bearing the galls. The extracts were then subjected to bioassay (against *P. tremulae*) guided separation. Initial experiments were carried out on sawdust from the black galls. Separate portions of the sawdust were extracted with ethyl acetate, dichloromethane (DCM), methanol, and water. The ethyl acetate and dichloromethane extracts were separated into acidic and non-acidic materials ($NaHC0_3$ extraction). Preliminary bioassays showed that these acidic extracts showed fungicidal activity against *P. tremulae*. The components present in the acidic fractions were separated, identified, and subjected to bioassay. The first objective was to identify the antifungal agent, which proved to be benzoic acid (BA), the second to monitor the level of this substance in the various wood substrates.

Materials and Methods

General methods

Gas chromatography was performed on a Varian 3700 gas chromatograph using a 50m.PONA (HP1) column, 0.21 mm x 0.5 μ film, flow rate at 55 psi= 0.6 mL/minute. Mass spectra were determined on an AEI-50 mass spectrometer. Nmr spectra (^1H and ^{13}C) were obtained on Bruker WM-360 or Varian Unity 500 multinuclear spectrometers. Chemical shifts are referenced to residual hydrogen (7.26 ppm) or carbon (77.0) absorption of $CDCl_3$. Flash chromatography was performed on silica gel 230-400 mesh, General Intermediates of Canada. Analytical tlc was carried out on E. Merck precoated aluminum sheets of Si gel 60 F 254. Ft-ir spectra were recorded on a Nicolet 7199 FTIR interferometer. All solvents were distilled prior to use.

Aspen and black galls

Aspen (*P. tremuloides*) trees, some with black galls, some with heartwood rot caused by *P. tremulae*, and some healthy trees, were collected from the same clone near Blue Ridge, Alberta in July, 1993. Black galls were excised with a chain saw. Clean wood from a galled tree was excised 25 cm above the gall. Clean wood from a *P. tremulae* infected tree was removed from above the decayed area. The wood was chipped the following day and chips that were not used immediately were frozen. The chips which were used for extraction were ground to sawdust and air dried to constant weight at room temperature.

Extraction, separation, and identification of acidic components of black galls.

Air-dried sawdust from black gall tissue (0.5 kg) was suspended in ethyl acetate (2 L) and vigorously stirred for 2 hours, then filtered. The filtered sawdust was recovered and the extraction repeated. The combined extracts were dried (Mg $S0_4$) and concentrated to about one-third volume, then extracted three times with sat. aq. $NaHC0_3$ (500 mL). The combined bicarbonate extracts were acidified with 2 N HCl, then back extracted with ethyl acetate (2 times, 1 L). Evaporation of the ethyl acetate provided the acidic metabolites (5.0 g). Evaporation of the original ethyl acetate solution (bicarbonate washed) provide non-acidic material (21.0 g).

The acidic material was separated by column chromatography over silica gel (150 g), eluting with hexane, then mixtures of hexane-ethyl acetate, then ethyl acetate. The fractions obtained were subjected to thin layer chromatography (tlc) and further purified by flash chromatography and preparative tlc where necessary. Benzoic acid (0.8 g) was eluted first, followed by *trans*-cinnamic acid (0.05 g), naringenin (**1**, 30 mg), 7'-methyl-3-hydroxynaringenin (**2**, 15 mg), *p*-hydroxybenzoic acid (30 mg), *p*-hydroxycinnamic acid (0.3 g), aromadendrin (**3**, 45 mg), and taxifolin (**4**, 12 mg). Benzoic acid, *trans*-cinnamic acid, *p*-hydroxybenzoic acid, and *p*-hydroxycinnamic acid were identified by comparison (mass spectra, **ir** spectra, ^1H nmr spectra) with authentic samples. Naringenin (**1**), aromadendrin (**3**), and taxifolin (**4**) were identified by comparison with published **ir**, nmr, and mass spectral data (see Harborne and Mabry 1982, for leading references). 7'-methyl-3-hydroxynaringenin (**2**) was identified by comparison with published **ir** and nmr data (Herz *et al.* 1972).

Bioassay methods

Bioassays of crude extracts and chromatographic fractions against *P. tremulae* were carried out on carrot agar diffusion plates as previously described (Chakravarty and Hiratsuka 1992). In this case, 10 mg of sample was suspended in 10 mL of distilled water and one mL of the sample was added to diffusion wells on seven day old cultures of *P. tremulae*. After four weeks incubation at 22°C in the dark, the zone of inhibition around the wells was measured. The bioassays on pure benzoic acid were carried out by incorporation of benzoic acid (100 ppm, 300 ppm, 500 ppm, 700 ppm, 900 ppm, 1000 ppm) into the agar at the time of preparation. The 100 and 300 ppm BA level showed no inhibition of growth of *P. tremulae* relative to controls, while the 900 and 1000 ppm level showed complete inhibition. The 500 and 700 ppm levels showed 25-30% inhibition.

Extraction with dichloromethane

The air-dried sawdust (50 g) was suspended in dicholoromethane (400 mL) and stirred for 2 hours, then filtered. The filtered sawdust was recovered and the extraction repeated twice more. The combined DCM filtrates were dried ($MgS0_4$) and concentrated to about one-third volume, then

extracted three times with sat.aq. $NaHC0_3$ (100 mL). The combined bicarbonate extracts were acidified (slowly) with 2N HCl, then back extracted with DCM (3 times, equal volumes). Evaporation of the acid extract provided the crude acids (0.43 g from black gall tissue, 0.2 g from clean wood of gall tree, 0.075 g from clean wood of non-galled healthy tree).

Analysis of benzoic acid content

The same extraction procedure as described above was used, except that *p*-toluic acid was added to the sawdust to serve as an internal standard to measure the extraction efficiency. Since the concentration of benzoic acid varied considerably according to the source of wood used, both the amount of *p*-toluic acid and the amount of sawdust were adjusted to give comparable gas chromatography traces. Preliminary tests involving the direct isolation of benzoic acid from the various types of substrate allowed us to estimate the approximate amount present. The crude DCM extract was concentrated and extracted with aq. $NaHC0_3$ as above and the total crude acids were liberated and back extracted into DCM. This DCM acid extract was concentrated to 30~40 mL and phenylacetic acid was added as an internal standard for the gc analysis. Control experiments showed that *p*-toluic acid and phenylacetic acid were not present among the acids extracted from the sawdusts. The DCM acid extract containing the internal standards was treated with diazomethane in DCM until no further evolution of nitrogen was observed. The volume of the ester solution was adjusted as necessary to obtain suitable intensity gc peaks and aliquots were subject to gc analysis using the following temperature program: 60° (15 minutes), 5°/minute increase for 44 minutes, final temperature 280°. Under these conditions, methyl benzoate appeared after 28.5 minutes, methyl phenylacetate after 31.6 minutes, and methyl *p*-toluate after 33.1 minutes. The peak areas were measured electronically and the amount of benzoic acid present in the sample was calculated according to the method of Morales (Morales *et al.*1992). The results are shown in Table 1.

Table 1. Analysis of benzoic acid in aspen.

Source	% benzoic acid	ppm, benzoic acid
black gall tissue	0.17	1700
clean wood, gall tree	0.0090	90
clean wood, healthy tree	0.0014	14
clean wood, *Phellinus* infected tree	0.0039	39

RESULTS AND DISCUSSION

The black gall tissue was extracted with ethyl acetate, dichloromethane, methanol, and water. The ir spectra of the crude ethyl acetate and DCM extracts clearly showed the presence of carboxylic acids, so these extracts were separated into acidic and non-acidic materials by extraction of the acids into aqueous bicarbonate solution. Bioassays of the various extracts against *P. tremulae* showed that the acidic portions of the ethyl acetate and DCM extracts displayed the strongest activity. The non-acidic material showed no activity, while the methanol and aqueous extracts possessed some activity. Bioassay of a DCM extract of the aqueous extract also indicated activity.

Since the acidic portion of the ethyl acetate fraction showed activity and tlc indicated that it contained more components than the DCM extract it was separated into its components by a combination of column chromatography, flash chromatography, and preparative tlc. The major component was benzoic acid (BA). Other components include *trans*-cinnamic acid, *p*-hydroxybenzoic acid, *p*-hydroxycinnamic acid, and the known flavonoids (Harborne and Mabry 1992) naringenin (**1**), 7'-methyl-3-hydroxynaringenin (**2**), aromandendrin (**3**), and taxifolin (**4**). Only BA showed activity (at 1000 ppm) against *P. tremulae*. Benzoic acid is known to have weak antimicrobial activity (Lueck 1980) and as such is widely used as a food preservative, often in the form of its more soluble sodium salt.

In order to determine whether the level of benzoic acid was significantly greater in trees bearing black galls than in other aspen we developed an analytical procedure for determining the amount of benzoic acid present in various aspen tissues. Since dichloromethane was as effective a solvent as ethyl acetate in extracting benzoic acid from sawdust, and since it is more volatile and extracted less of the more polar metabolites, it was chosen for the analytical study. Sawdust from black gall tissue, from trees carrying black galls, from healthy non-galled trees, and from *P. tremulae* infected trees, was extracted with dichloromethane and the benzoic acid level (as methyl benzoate) was determined by gas chromatography as described above. The results of this study are shown in Table 1. The black gall tissue contained much more benzoic acid than the clean wood from the galled tree, but this in turn showed an appreciably higher level of benzoic acid than did the clean wood from a healthy tree. The clean wood from the *P. tremulae* infected tree had a concentration of benzoic acid intermediate between that of the galled tree and the clean tree (Table 1).

Our bioassays indicate that the minimum inhibitory concentration of benzoic acid against *P. tremulae* is in the range 800-1000 ppm. Since the concentration of benzoic acid in the galled tree is only about 90 ppm, it seems unlikely that benzoic acid is the direct cause of the "black gall effect".

It has previously been reported that the bark of aspen contains the glucosides of benzoic and salicylic acids (Hubbes 1969). It is known that in some plants salicylic acid plays an important role in the induction of resistance to pathogens, so-called systemic acquired resistance (SAR) (Yalpani and Raskin 1993; Horvath and Chua 1994.) Salicylic acid is a signal compound that induces production of so-called pathogenesis-related (PR) proteins, which function to restrict the progress of infecting pathogens (reviewed in Horvath and Chua 1994). Salicylic acid is produced by the monooxygenase catalyzed oxidation of benzoic acid (Leon *et al.* 1993; Yalpani *et al.* 1993). Benzoic acid is itself biosynthesized from phenylalanine *via trans*-cinnamic acid (Yalpani *et.al.* 1993), one of the minor

acids isolated from the black gall tissue. Although salicylic acid was not isolated directly from the black galls, it was detected in the form of its glycoside (which is not soluble in dichloromethane), by extraction of the sawdust remaining from the dichloromethane treatment with butyl alcohol. Acid hydrolysis of the butyl alcohol extract provided both salicylic and benzoic acids, indicating that both were present as glycosides in the black gall tissue.

It is recognized that the level of any signalling molecule in the cell must be carefully regulated and in the case of tobacco, that very low levels of salicylic acid are found in healthy leaves, but that the levels increase dramatically after infection, as do the levels of benzoic acid (Yalpani *et al.* 1993). It is possible to speculate that the "black gall effect" may be indirectly related to the increased concentration of benzoic acid in the gall trees. In tobacco, the hydroxylase required to convert benzoic acid to salicylic acid is activated by the pathogen (Yalpani *et al.* 1993). If a similar defence system exists in aspen, salicylic acid induced SAR may be facilitated by the ready availability of the salicylic acid precursor.

We have seen (Table 1) that aspen infected by *P. tremulae* have a somewhat elevated level of benzoic acid, perhaps as a response to the invasion of the pathogen. It has been shown that several *Phellinus* species are very proficient in methylating aromatic carboxylic acids (Harper *et al.* 1989). The odor of methyl salicylate (wintergreen) has long been associated with *Phellinus* heartwood rot of aspen (Collins and Halim 1972), and we have shown by ^{13}C-labelling experiments that *P. tremulae* metabolizes benzoic acid, *via* methyl benzoate and 2-carbomethoxyoxepin (**5**), to methyl salicylate (Ayer and Cruz 1995). Since methyl salicylate, unlike salicylic acid, does not appear to induce SAR in plants (Yalpani and Raskin 1993), the methylating ability of *Phellinus* may be a factor in its successful invasion of aspen. The black gall trees, with their higher level of the precursor of salicylic acid, may be rendered more resistant to the pathogen.

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Appendix 5

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Antagonism of two decay fungi, Peniophora polygonia and Phellinus tremulae associated with Populus tremuloides

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Abstract

Interactions between the two most common decay fungi of *Populus tremuloides*, *Peniophora polygonia* and *Phellinus tremulae* were studied. *Pe. polygonia* inhibited the growth of *Ph. tremulae* when grown in paired culture. The presence of a culture of *Pe. polygonia* reduced the growth of *Ph. tremulae* in a volatile metabolite test. A culture filtrate of *Pe. polygonia* reduced the growth of *Ph. tremulae* in liquid culture. The growth of both the fungi was inhibited, and they formed a dark brown line at contact point when grown on a *Po. tremuloides* block.

Key words: *Peniophora polygonia* – *Phellinus tremulae* – *Populus tremuloides* – Antagonism – Fungal metabolites.

1 Introduction

Trembling or quaking aspen (*Populus tremuloides* Michx.) is the most widely distributed tree species in North America. It is a short-lived, pioneer species which grows rapidly on a wide range of soil and site conditions (STROTHMAN and ZASADA 1957). In Canada, aspen represents 54% of net merchantable hardwood timber and 11% of the entire Canadian net timber resources (HUNT et al. 1978). In the province of Alberta, aspen comprises about 40% of total forest resources (HIRATSUKA and LOMAN 1984; BRECK 1987; ONDRO 1989).

Decay and stain caused by various fungi have been identified as the two most important factors limiting the utilization of aspen (HIRATSUKA et al. 1990). The economic consequences of the types and degree of decay and stain differ significantly according to the end use. More than 250 species of fungi have been associated with decay in aspen (LINDSEY and GILBERTSON 1978), and many other non-decay fungi have been isolated from decayed, stained, and clear aspen wood (GOOD and NELSON 1962; SHIGO 1967; LAFLAMME and LORTIE 1973; GINNS 1986).

Phellinus tremulae (Bond.) Bond. et Borisov is a serious wood destroying fungus occouring on different species of *Populus* (SCHRENK and SPAULDING 1909; BASHAM 1958; CERNY 1972; WIKSTRÖM 1976; WIKSTRÖM and UNESTAM 1976). In Alberta, about 39% of trunk decay volume is caused by this fungus (THOMAS et al. 1960). BASHAM (1958) reported that old stands of *Populus tremuloides* Michaux contained higher proportions of decayed heartwood than younger ones. According to him, *Ph. tremulae* invades the tree only after the invasion of some preliminary fungi which do not decay the wood but alter it in some way that makes it possible for *Ph. tremulae* to establish itself afterwards. SHIGO (1965, 1963) investigated the discolorations around rot columns caused by *Fomes ignarius* (L.: Fr.) Kickx (= *Ph. tremulae*) in some species and found that bacteria and non-hymenomycetous fungi are always associated with it.

The second most prevalent cause of decay in *Populus* spp. is *Peniophora polygonia* (Persoon: Fries) Bourdot et Galzin. This fungus does not cause large columns of advanced decay as does *Ph. tremulae* but is more often found in decayed and discolored wood than

Ph. tremulae (HIRATSUKA et al. 1990). The decay caused by *Pe. polygonia* is acceptable for the pulp and paper industry, and requires less bleaching than does wood decayed by *Ph. tremulae* (PAPRICAN, personal communication). NAVRATIL and WINSHIP (1978) suggested that *Ph. tremulae* and *Pe. polygonia* are exclusive or antagonistic to each other. HIRATSUKA et al. (1990) reported that most of the trees with decay caused by *Pe. polygonia* do not have white spongy decay caused by *Ph. tremulae*, and in trees where both the fungi coexist, there are clear demarcation lines between the areas infected by each organism. Although *Pe. gigantea* (Fr.) Massee has been suggested as a biological control agent against certain decay fungi (RISHBETH 1963; GREIG 1976a, b; PARKER 1977), no information is available for *Pe. polygonia*.

The objectives of this investigation were to study the interactions *in vitro* between *Pe. polygonia* and *Ph. tremulae* in agar plates and *Po. tremuloides* blocks and to determine the effect of the culture filtrate of *Pe. polygonia* on the growth of *Ph. tremulae*.

2 Materials and methods

2.1 Organisms

Two fungal isolates, *Phellinus tremulae* (NOF 1464) and *Peniophora polygonia* (NOF 1494), obtained from basidiomes associated with *Populus tremuloides*, were used in this study. They were deposited in the fungal culture collection at Northern Forestry Centre, Forestry Canada, Edmonton, Alberta (NOF) and were maintained at 5 °C on carrot agar (CA) medium in McCartney tubes until used.

2.2 Antagonism in vitro

Antagonism of *Ph. tremulae* and *Pe. polygonia* was studied on malt extract agar (MEA), potato dextrose agar (PDA), and CA media in 90 mm Petri plates. *Ph. tremulae* (a slow growing fungus) was inoculated with 5 mm agar plugs (taken from the periphery of 2 week old mycelial mats grown in the dark) at the margin of the plate and allowed to grow at 22 °C in the dark. Seven days later, 5 mm mycelial disks of *Pe. polygonia* from agar cultures were placed on the plate opposite *Ph. tremulae* and were then incubated as described above. The inhibition zone formed around *Ph. tremulae* colony was measured 15 days later.

Similarly, antagonism of *Ph. tremulae* and *Pe. polygonia* was studied on popsicle stick (8.5×0.3 cm, manufactured by U.S. Stick, WI, U.S.A.). The sticks were dipped overnight in liquid malt extract (ME), carrot extract (CE) and potato dextrose broth (PDB). After autoclaving for 45 min at 121 °C, the sticks were placed on 90 mm Petri plates containig MEA, PDA, and CA media. *Ph. tremulae* was inoculated (5 mm mycelial agar plugs) at one end of the stick and the plates were incubated at 22 °C in the dark. After 7 days, 5 mm mycelial agar plugs of *Pe. polygonia* were inoculated at the other end of the stick. The plates were returned to the incubator. The inhibition zone formed around *Ph. tremulae* colony was recorded 15 days later.

2.3 Volatile antibiotic test

The effect of volatile substances produced by *Pe. polygonia* on the growth of *Ph. tremulae* was studied in a modification of the method described by YAMAOKA and HIRATSUKA (1992). Inoculum discs of *Pe. polygonia* were placed at the centre of Petri plates (90 mm diam.) containing MEA, PDA, and CA media. The plates were incubated at 22 °C in the dark. After 7 days, a second set of Petri plates were inoculated with 5 mm mycelial plugs of *Ph. tremulae* in MEA, PDA, and CA media. Immediately after inoculation, the upper

lids of the Petri plates containing *Ph. tremulae* and *Pe. polygonia* (7 day old culture) were removed and the two culture plates joined on top of each other leaving a space remained between their respective cultures. The joined plates were wrapped with parafilm and incubated as described above. Control plates were prepared in the same way with nutrient media lacking *Pe. polygonia*. Colony diameters of *Ph. tremulae* were measured 25 days later.

2.4 Effect of the culture filtrate of Pe. polygonia on the in vitro mycelial growth of Ph. tremulae

Pe. polygonia was grown on liquid ME, CE, and PDB at 22°C in the dark. After 28 days, the mycelium was harvested on Whatman No. 1 filter paper. The culture filtrate was collected and stored at 5°C in the dark for 12 hours. Fifty ml quantities of liquid media (ME, CE, and PDB) were autoclaved in 250 ml flasks for 15 min at 121°C, aseptically inoculated with 5 agar plugs (5 mm diam.) of actively growing mycelium of *Ph. tremulae*. The agar was removed with a sterile scalpel and only the mycelial mat was inoculated into the flask. Five ml of culture filtrate of *Pe. polygonia* was then added to each flask. The control consisted of 5 ml of sterile distilled water. The flasks were kept in the dark on a shaker at room temperature (22 ± 2°C). After the 28 day incubation period, the mycelia of *Ph. tremulae* were harvested on a Whatman No. 1 filter paper, ovendried at 70°C for 48 hours, and the dry weight of mycelia calculated.

The effect of the culture filtrate of *Pe. polygonia* on *Ph. tremulae* was also studied on agar diffusion plates. These plates were prepared from 90mm plastic Petri plates containing 40 ml of CA medium, by removing 5-mm diameter agar plugs from each of four quarters of the plate. Five mm-diameter agar plugs of *Ph. tremulae* was inoculated in the centre of the agar diffusion plate, and incubated at 22°C in the dark. The culture filtrate of *Pe. polygonia* was prepared by filtering 15 day old liquid culture (grown on CE medium in 250 ml flask) through both a Whatman No. 1 filter paper and a 0.45 μm Millipore filter and then drying it on a rotary evaporator at 45°C. The evaporated sample was resuspended in 5 ml of distilled water. One ml of the concentrated culture filtrate (filter sterilized) of *Pe. polygonia* was added to diffusion wells of each of the ten replicate plates containing seven day old culture of *Ph. tremulae*. The plates were then incubated as described above. After incubation for 25 days, the zone of inhibition around each of the diffusion wells was recorded. Microscopic observations of hyphae were also made.

2.5 Inoculation test on aspen blocks

For this experiment, healthy and decayed (caused by *Ph. tremulae*) aspen trees were felled and collected. Small blocks (8×4 cm) were cut from the wood. A hole (10 mm) was drilled from opposite sides of each block. All blocks were autoclaved for 2 hours at 121°C. Undecayed blocks were inoculated with *Pe. polygonia* and *Ph. tremulae* (grown on aspen saw dust for 30 days) on opposite holes. In *Ph. tremulae* infected blocks, inoculum of *Pe. polygonia* was introduced into the undecayed section. All holes were sealed with wax (Tissue Prep 2, Fisher Scientific, NJ, U.S.A.) and the blocks were incubated in a glass jar containing a small volume of 1.5% water agar (to maintain humidity of the block). The lid of the jar was sealed with parafilm and kept in room temperature (22 ± 2°C) in dark. After the 120 days incubation period, the blocks were removed from the jars, washed with water, and then split in half. The inhibition line, where *Ph. tremulae* and *Pe. polygonia* contacted each other, was recorded and photographed.

2.6 Statistical analysis

Data were subjected to analysis of variance. The individual means were compared using Scheffe's test for multiple comparison using SAS software (SAS INST. INC. 1985).

3 Results

3.1 Antagonism in vitro

Growth of *Ph. tremulae* was significantly inhibited by *Pe. polygonia* when grown in paired culture. Among three different nutrient media tested, CA medium was found to be the best for growth of both fungi. The inhibition zone, which ranged from 1–4 mm, formed around the colony of *Ph. tremulae*, was observed on all three nutrient media tested (Table 1, Fig. 1A). Similarly, antagonism of *Pe. polygonia* towards *Ph. tremulae* was also observed on the popsicle stick. There was a clear demarcation line where both the fungi grew in close proximity to each other (Fig. 1B).

Table 1. Radial growth of Ph. tremulae after 15 days incubation on agar media and resulting antagonistic effect by Pe. polygonia

Fungus	Agar media	Radial growth (mm)	Inhibition zone (mm)
Ph. tremulae	MEA	12.5c	1.0c
	PDA	17.0b	2.5b
	CA	25.0a	4.0a

Values are the means of 20 replicates. Means followed by the same letters in columns are not significantly (P = 0.05) different from each other by Scheffe's test. MEA = malt extract agar, PDA = potato dextrose agar, CA = carrot agar.

3.2 Volatile antibiotic test

In the volatile metabolite test, the presence of a culture of *Pe. polygonia* significantly inhibited the growth of *Ph. tremulae*. The inhibition was observed in all three media tested (Table 2). The growth of *Ph. tremulae* was reduced 10, 22.2, and 26.6% in MEA, PDA, and CA media respectively from control (Table 2).

Table 2. Effect of volatile antibiotics produced by Pe. polygonia on the growth of Ph. tremulae

Agar media	Colony diameter (mm) of *Ph. tremulae*		
	Control (without *Pe. polygonia*)	With *Pe. polygonia*	Reduction from control (%)
MEA	10.0a	9.0a	10.0
PDA	22.5a	17.5b	22.2
CA	30.0a	22.0b	26.6

Values are the means of 20 replicates. Means followed by the same letters in rows are not significantly different from each other by Scheffe's test. MEA = malt extract agar, PDA = potato dextrose agar, CA = carrot agar.

3.3 Effect of the culture filtrate of Pe. polygonia on the in vitro growth of Ph. tremulae

The mycelial growth of *Ph. tremulae* was inhibited when treated with culture filtrate of *Pe. polygonia* in agar diffusion plates (Fig. 1D). Similarly, mycelial growth of *Ph. tremulae* in liquid culture was reduced significantly when treated with the culture filtrate of *Pe. polygonia* (Table 3). The maximum inhibition of growth was recorded 4 weeks after inoculation (Table 3).

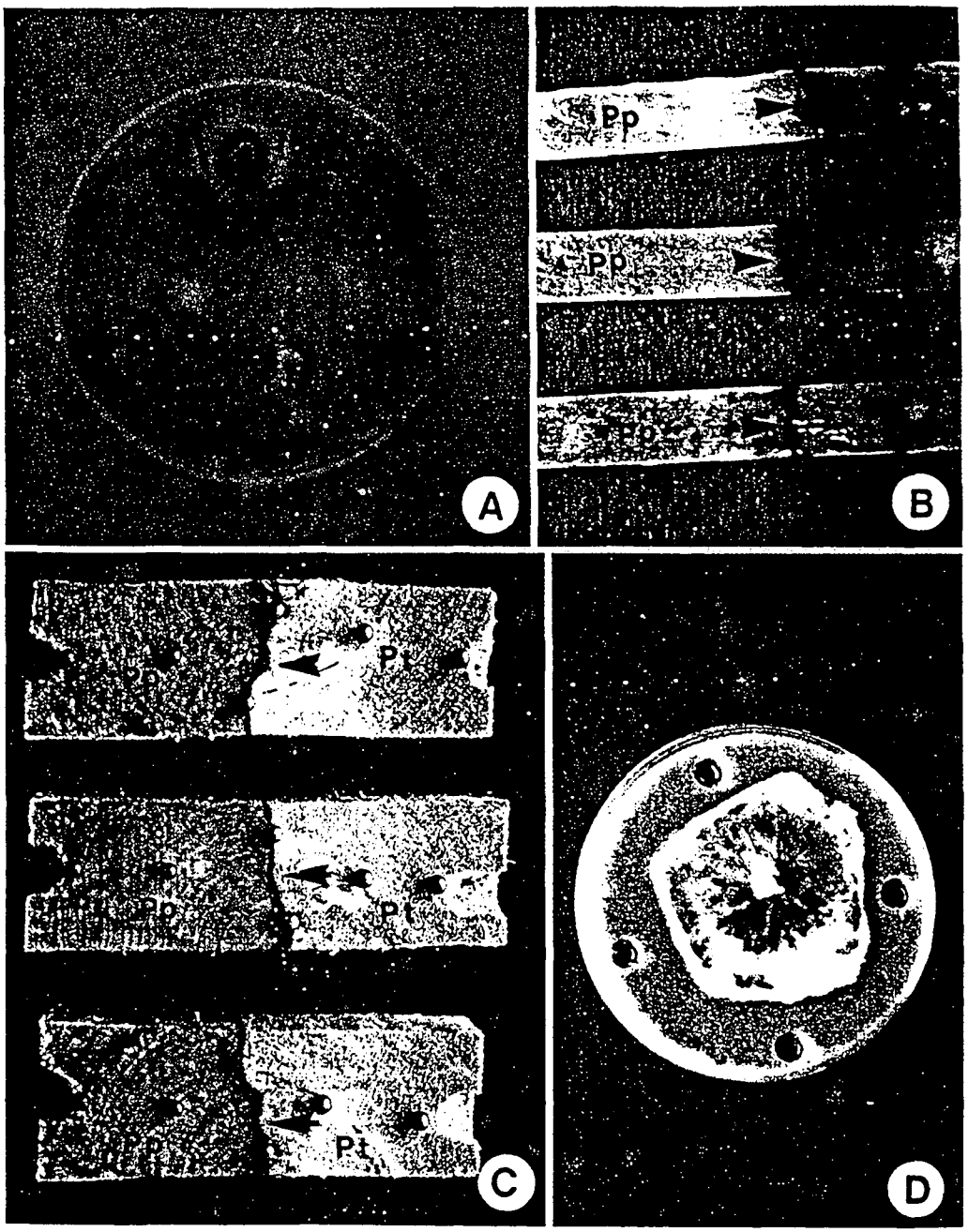

Fig. 1. Antagonism of *Pe. polygonia* (Pp) and *Ph. tremulae* (Pt) on carrot agar medium (A) and on the popsicle sticks (B). Arrow denotes demarcation line where the fungi came into contact with each other. C = Interaction between *Pe polygonia* (Pp, blue pins) and *Ph. tremulae* (Pt, red pins) on aspen block. Arrow and green pins denote demarcation line where the fungi came into contact with each other. D = Inhibition of *Ph. tremulae* mycelia on agar diffusion wells when treated with culture filtrate of *Pe. polygonia*

Table 3. Effect of culture filtrate of Pe. polygonia on the in vitro mycelial growth (mg, dry wt.) of Ph. tremulae

Inoculation period (week)	Mycelial dry wt. (mg) of *Ph. tremulae*					
	Control			Culture filtrate of *Pe. polygonia*		
	ME	PDB	CE	ME	PDB	CE
1	15.5c	30.0b	49.9a	14.5c	30.5b	48.7a
2	20.0e	55.0c	75.0a	22.5e	48.3d	68.4b
3	45.6d	70.0b	96.0a	38.0e	61.5c	70.9b
4	62.5e	85.5c	108.3a	49.5f	69.5d	71.0b

Values are the means of 20 replicates. Means followed by the same letters in a row are not significantly (P = 0.05) different from each other by Scheffe's test. ME = malt extract, PDB = potato dextrose broth, CE = carrot extract.

3.4 Inoculation test on aspen blocks

Growth of both fungi was inhibited when they confronted each other on the aspen block (Fig. 1C). There was a dark brown demarcation line where the fungi contacted each other (Fig. 1C).

4 Discussion

Fungi have been increasingly used in recent years to control plant diseases incited by other fungi (BAKER and COOK 1982; COOK and BAKER 1983). In most of the cases, control of the plant pathogens is effected through parasitism. This may take the form of surface parasitism without penetration of the host fungus, surface contact followed by penetration and formation of intercellular hyphae within the host hyphae. Alternatively, pathogens may be controlled by the production of antifungal compounds by the antagonist fungus.

In our experiment, *Pe. polygonia* was found to be an effective antagonist towards *Ph. tremulae*. Mycelial growth of *Ph. tremulae* in both agar and liquid culture was inhibited by *Pe. polygonia*. Swelling and vacuolation of the hyphae in *Ph. tremulae* were also observed under a microscope from the agar diffusion test. The *Pe. polygonia* isolate used in this study was obtained from a naturally infected *Po. tremuloides* tree. When inoculated on *Po. tremuloides* blocks, growth of both fungi was inhibited when they came into contact with each other, i. e. *Pe. polygonia* prevented further extension of *Ph. tremulae* on wood blocks and *vice versa*. There was a dark brown demarcation line where the fungi contacted each other. HIRATSUKA et al. (1990) reported that *Pe. polygonia* can compete effectively against *Ph. tremulae* for infection sites in naturally occurring *Po. tremuloides* trees. They also found that most of the trees infected by *Pe. polygonia* lack *Ph. tremulae* decay and in trees where both the fungi co-exist, there are clear demarcation lines between areas infected by each organism. Our laboratory studies with *Po. tremuloides* blocks also revealed similar findings. Although the mechanism(s) of this antagonism is not yet known, our results indicate that *Pe. polygonia* produced antifungal compound(s) which inhibited the growth of *Ph. tremulae*.

Several investigators have proposed the use of another species of *Peniophora*, *Pe. gigantea* (Fr.) Massee [= *Phlebiopsis gigantea* (Fr.) Jülich], for biological control of a decay fungus, *Fomes annosus* (Fr.) Cke. [= *Heterobasidium annosum* (Fr.) Bref.] (RISHBETH 1963; KUHLMAN and HENDRIX 1964; BOYCE 1966; ARTMAN and STAMBUGH 1970; GREIG 1976a). Artificial inoculation on pine stumps with oidia of *Pe. gigantea* greatly reduced colonization of pine stumps by *F. annosus*, and thus contributed to the biological control of decay. WEBB (1973) found that the inoculation of pine stumps with *Pe. gigantea* was as

effective as chemical treatment. *Pe. polygonia* has not yet been used to control *Ph. tremulae*. The growth of *Pe. polygonia* in *Po. tremuloides* trees might restrict the extension of *Ph. tremulae* by occupying the available food base. Since *Pe. polygonia* has limited pathogenicity in *Po. tremuloides* trees, it may serve as a desirable agent for the biological control of *Ph. tremulae*.

The efficacy of the compounds produced by *Pe. polygonia* on the growth inhibition of *Ph. tremulae* suggests that *Pe. polygonia* does produce antifungal metabolites. To identify these compounds, further chemical analysis of the culture filtrate and mycelia of *Pe. polygonia* are in progress.

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Summary

Phellinus tremulae and *Peniophora polygonia* are two important decay fungi on *Populus tremuloides*. *Pe. polygonia*, however, does not cause large columns of advanced decay as does *Ph. tremulae*. The decay caused by *Pe. polygonia* is acceptable by the pulp and paper industry. These two fungi are mutually exclusive or antagonistic to each other. In paired culture, growth of *Ph. tremulae* was reduced significantly by *Pe. polygonia*. *Pe. polygonia* inhibited the growth of the colonies of *Ph. tremulae* by producing volatile metabolites. The culture filtrate of *Pe. polygonia* also inhibited the mycelial growth of *Ph. tremulae* in liquid culture. In *Po. tremuloides* blocks, *Pe. polygonia* prevented further extension of *Ph. tremulae* and there was a clear demarcation line where the fungi came into contact with each other.

Résumé

Antagonisme entre deux champignons lignivores du Populus tremuloides: Peniophora polygonia et Phellinus tremulae

P. tremulae et *P. polygonia* sont deux champignons lignivores, importants sur *P. tremuloides*. *P. polygonia* ne provoque cependant pas de grandes colonnes d'altération intense comme le fait *P. tremulae*. L'altération due à *P. polygonia* reste acceptable pour l'industrie de la pulpe et du papier. Ces deux champignons s'excluent mutuellement ou sont antagonistes. En cultures doubles, la croissance de *P. tremulae* est significativement réduite par *P. polygonia*. L'inhibition est due à la production de métabolites volatiles. Les filtrats de culture de *P. polygonia* inhibent également la croissance mycélienne de *P. tremulae* en milieu liquide. Dans des blocs de bois de *P. tremuloides*, *P. polygonia* empêche l'extension de *P. tremulae* et il y a une nette ligne de démarcation au contact entre les deux champignons.

Zusammenfassung

Antagonismus zwischen zwei holzzerstörenden Pilzen von Populus tremuloides, Peniophora polygonia und Phellinus tremulae

Phellinus tremulae und *Peniophora polygonia* sind zwei wichtige holzzerstörende Pilze auf *Populus tremuloides*. Im Gegensatz zu *P. tremulae* bildet *P. polygonia* keine ausgedehnten Bereiche fortgeschrittenen Abbaus. Daher wird durch *P. polygonia* befallenes Holz von der Papierindustrie akzeptiert. Die beiden Pilze schließen sich gegenseitig aus, sie verhalten sich als Antagonisten. Das Wachstum von *P. tremulae* wurde in Dualkulturen signifikant gehemmt. *P. polygonia* hemmte das Wachstum von Kolonien von *P. tremulae* mittels flüchtiger Metabolite. Kulturfiltrate von *P. polygonia* hemmten das Myzelwachstum von *P. tremulae* auch in Flüssigkultur. Auf Holzstücken von *Populus tremuloides* verhinderte *P. polygonia* eine weitere Ausbreitung von *P. tremulae*. Dort, wo sich die beiden Pilzmyzelien im Holz trafen, bildete sich eine deutliche Demarkationslinie.

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Role of *Sporormiella similis* as a potential bioprotectant of *Populus tremuloides* wood against the blue-stain fungus *Ophiostoma piliferum*

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Role of *Sporormiella similis* as a potential bioprotectant of *Populus tremuloides* wood against the blue-stain fungus *Ophiostoma piliferum*

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Interactions between *Sporormiella similis* Khan & Cain and the blue-stain fungus *Ophiostoma piliferum* (Fr.) H. & P. Sydow, both isolated from *Populus tremuloides* Michx. wood, were investigated. *Sporormiella similis* significantly reduced the growth of *O. piliferum* in vitro when grown in dual culture, in addition to inhibiting the growth of *O. piliferum* on agar media and in liquid culture when treated with a culture filtrate of *S. similis*. *Ophiostoma piliferum* failed to colonize *P. tremuloides* wood chips when they were preinoculated with *S. similis*. Ten known compounds were isolated and identified from the culture filtrate of *S. similis*. These compounds showed varied fungitoxic effect against *O. piliferum* at concentrations of 1 to 1000 μg/mL. The potential for *S. similis* as a biological control agent against *O. piliferum* on *P. tremuloides* is discussed.

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Nos travaux ont porté sur les interactions entre *Sporormiella similis* Khan & Cain et un champignon responsable du bleuissement, *Ophiostoma piliferum* (Fr.) H. & P. Sydow, tous deux isolés à partir de bois de *Populus tremuloides* Michx. In vitro et en culture mixte, *S. similis* a significativement réduit la croissance de *O. piliferum*, et a inhibé sa croissance sur gélose et en bouillon de culture après traitement avec un filtrat de culture de *S. similis*. *Ophiostoma piliferum* n'est pas parvenu à coloniser des copeaux de *P. tremuloides* après qu'ils aient été inoculés avec *S. similis*. Dix composés connus ont été isolés et identifiés dans le filtrat de culture de *S. similis*. Ces composés révélaient à divers degrés un effet fongitoxique contre *O. piliferum* en concentration comprise entre 1 et 1000 μg/mL. On étudie le potentiel de *S. similis* à titre d'agent de lutte biologique contre *O. piliferum* sur *P. tremuloides*.

Introduction

Trembling aspen (*Populus tremuloides* Michx.) has in recent years become increasingly important as a source of pulp for the paper-making industry in western Canada (Hiratsuka et al. 1990). Sap stain in harvested logs or in wood chips, however, increases the cost of the final product owing to the increased use of chemical bleaches. Also, by-products from the bleaching process are detrimental to the environment, as are the chemical wood preservatives such as sodium pentachlorophenol or sodium tetrachlorophenol by which fungal stain can be prevented. These protective chemicals are toxic and persist in the environment for a long period of time. An environmentally friendly alternative to chemical preservatives may be the use of biological protection to prevent fungal stain on chips or logs. Such recent studies with other tree species have shown some promising results (Kreber and Morrell 1993; Seifert et al. 1987, 1988).

As part of a survey of microorganisms occurring naturally in aspen, several fungi with potential as bioprotectants of aspen wood against stain-causing fungi were identified and their metabolites were characterized (Chakravarty and Hiratsuka 1994; Hiratsuka et al. 1994; Ayer and Miao 1993, 1994). The most active antagonistic fungus against the common blue-stain fungus *Ophiostoma piliferum* (Fr.) H. & P. Sydow is an isolate of *Sporormiella similis* Khan & Cain, which was recovered from the bark of a black gall on aspen trees in Alberta and northeastern British Columbia, Canada. Species of *Sporormiella* (=*Preussia* sensu von Arx 1973) are considered primarily as coprophilous (dung-inhabiting) fungi (Cain 1961; Ahmed and Cain 1972; Khan and Cain 1979; Valldosera and Guarro 1990), but increasingly they are becoming recognized as common endophytic inhabitants of woody substrates (Petrini 1984; Fisher et al. 1986, 1992; Petrini and Fisher 1988; Petrini and Müller 1979; Luginbühl and Müller 1980; Kowalski and Kehr 1992; Bills and Polishook 1991; Savonmäki et al. 1992). While their microbial interactions with other organisms are little known,

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isolates of *Sporormiella* spp. from dung have recently been shown to produce antibiotic compounds (Weber et al. 1990, 1992; Weber and Gloer 1991; Bergstrom et al. 1993).

In this study we examine the effectiveness of an isolate of *S. similis* recovered from wood as a potential bioprotectant against stain caused by *O. piliferum* on aspen wood chips and identify the compounds produced by *S. similis* that are responsible for bioprotection.

Materials and methods

Organisms

Two fungal isolates, *S. similis* (NOF 1639) and *O. piliferum* (NOF 1929), obtained from *P. tremuloides*, were used in this study. The isolates were deposited in the fungal culture collection at Northern Forestry Centre, Canadian Forest Service, Edmonton, Alberta, Canada (NOF) and were maintained at 5°C on malt extract agar (MEA) medium in McCartney bottles until used.

Antagonism of S. similis *against* O. piliferum

Antagonism of *S. similis* against *O. piliferum* in dual culture was studied on MEA, potato dextrose agar (PDA), and carrot agar (CA) in 90-mm Petri plates. Each plate was inoculated at the edge with 5 mm diameter agar plugs of *S. similis* (a slow-growing fungus) taken from the periphery of 10-day-old mycelium grown in the dark, and allowed to grow at 22°C in the dark. Seven days later, 5 mm diameter mycelial plugs of *O. piliferum* were inoculated on the plates opposite *S. similis* and incubated as described above. The inhibition zones formed around each *O. piliferum* colony were recorded and photographed.

Effect of culture filtrates of S. similis *on the growth of* O. piliferum

Sporormiella similis was grown on liquid malt extract in a shaker at room temperature (22 ± 2°C) in the dark. After a 25-day incubation period, the mycelium was harvested on Whatman No. 1 filter paper and the culture filtrate was collected. Fifty-millilitre quantities of culture filtrate were then dried on a rotary evaporator at 45°C. The evaporated samples were resuspended in 5 mL distilled water. The culture filtrate of *S. similis* was tested on agar diffusion plates and also on the in vitro mycelial growth of *O. piliferum*. Agar diffusion plates were prepared from 90-mm plastic Petri plates containing 40 mL of MEA medium by removing 5 mm diameter agar plugs from each of four quarters of the plate. A 5 mm diameter agar plug of *O. piliferum* was inoculated in the centre of each agar diffusion plate and incubated at 22°C in the dark. One millilitre of filter-sterilized culture filtrate of *S. similis* was added to diffusion wells of each of 15 replicate plates containing a 3-day-old culture of *O. piliferum*. The plates were incubated as described above. After a 10-day incubation period, the zone of inhibition around each of the diffusion wells was recorded and photographed.

To study the effect of the culture filtrate of *S. similis* on the mycelial production of *O. piliferum*, *S. similis* was grown in liquid malt extract at 25°C in the dark. After 10 days, the mycelium was harvested on Whatman No. 1 filter paper and the filtrate was collected. *Ophiostoma piliferum* was grown in twenty 250-mL flasks each containing 50 mL autoclaved liquid malt extract. *Ophiostoma piliferum* was inoculated as mycelial plugs (5 mm diameter) from actively growing mycelium. Five millilitres of *S. similis* culture filtrate was added to each flask. In the case of the control, 5 mL sterile distilled water was added. The flasks were kept in the dark on a shaker at room temperature (22 ± 2°C). After a 1-, 2-, 3-, or 4-week incubation period, the mycelium of *O. piliferum* was harvested on a Whatman No. 1 filter paper, oven-dried at 70°C for 48 h, and the dry weight of mycelium was measured.

Interactions of S. similis *and* O. piliferum *on* P. tremuloides *wood chips*

Populus tremuloides wood chips (150 g) were soaked in malt extract in each of forty 250-mL flasks. After 1 h, the malt extract was drained and the flasks were autoclaved for 80 min at 121°C. When cooled, 20 flasks were aseptically inoculated with five agar plugs (5 mm diameter) of actively growing mycelium of *S. similis*. Ten flasks were similarly inoculated with *O. piliferum*, and 10 control flasks were inoculated with sterile agar plugs of MEA. The flasks were incubated at 25°C in the dark. The flasks were periodically shaken to fragment and mix the growing mycelia. After 30 days, 10 flasks containing *S. similis* were inoculated with five mycelial plugs (5 mm diameter) of *O. piliferum* and returned to the incubator. Four treatments resulted: (*i*) *S. similis*, (*ii*) *O. piliferum*, (*iii*) *S. similis* + *O. piliferum*, and (*iv*) control. After 50 days of incubation, the wood chips were removed from the flasks and photographed.

Isolation of metabolites from the culture filtrate of S. similis

Sporormiella similis was grown in liquid malt extract medium (12 L) at 25°C in a shaker. After 25 days, the mycelium was harvested on Whatman No. 1 filter paper. The filtrate was extracted with ethyl acetate, and the crude extract was subjected to silica gel flash chromatography with petroleum ether and ethyl acetate to afford seven fractions. Fractions 2, 3, 4, and 5 were pure *p*-hydroxybenzaldehyde (1.1 mg), 3-methylindolin-2-one (2.1 mg), genistein (3.2 mg), and 2-(*p*-hydroxyphenyl)ethanol (8 mg), respectively. Fraction 1 was subjected to preparative thin-layer chromatography to afford 2-phenylethanol (11 mg), while fractions 6 and 7 gave phenylacetic acid (30 mg) and *p*-hydroxybenzoic acid (8 mg).

Similarly, *S. similis* was grown in 3 L liquid soybean medium (10 g of defatted soybean flour (type 1, Sigma), 10 g of glucose, 5 g of NaCl, 1 g of $CaCO_3$, and 1000 mL distilled water) at 25°C in a shaker. After 25 days, the mycelium was harvested on Whatman No. 1 filter paper and the filtrate was extracted with ethyl acetate. The crude ethyl acetate extract was subjected to silica gel flash chromatography with petroleum ether and ethyl acetate, petroleum ether + ethyl acetate + methyl alcohol, and methylene chloride + methyl alcohol to give five fractions. Fraction 1 was shown (gas chromatography (GC), GC – mass spectrometry, and GC – image resonance (IR)) to contain isobutyric acid (100 mg), 2-methylbutanoic acid (52 mg), 3,3-dimethylacrylic acid (2 mg), and tiglic acid (2 mg). Fraction 2 contained isobutyric acid (100 mg), isovaleric acid (50 mg), and 2-methylbutanoic acid (52 mg). Fraction 3 was recrystallized to afford pure anthranilic acid (11 mg). The mother liquor was shown (proton-NMR, GC) to contain phenylacetic acid (47 mg). Fraction 4 was subjected to preparative thin-layer chromatography (TLC) to afford phenylacetic acid, 2-(*p*-hydroxyphenyl)-ethanol (4 mg), and maltol (2.5 mg). In the same way fraction 5 gave phenylacetic acid and *p*-hydroxybenzoic acid (20 mg). The amount of anthranilic acid significantly decreased with age of the stock culture. Old cultures not producing anthranilic acid were found to contain lactic acid. All the compounds were identified by comparison with authentic samples using proton NMR, 1H-NMR, TLC, and IR.

Effect of metabolites of S. similis *on the in vitro growth of* O. piliferum

Ophiostoma piliferum was grown on Multiwell™ tissue culture plates (1.8 cm × 1.5 cm, diam. × length, individual wells; manufactured my Becton Dickinson Labware, Lincoln Park, N.J., U.S.A.). Two millilitres of MEA medium was poured into each well. When cooled, 25-µL solutions of each of the above compounds obtained from the culture filtrate of *S. similis* and also the sodium salts of isobutyric, isovaleric, 2-methylbutanoic acid, and phenylacetic acids at 1, 10, 100, and 1000 µg/mL in acetone were added separately to the surface of the agar. There were 20 replicates for each concentration. In the case of the control, 25 µL of acetone was added to each well. *Ophiostoma piliferum* was then inoculated as 5-mm agar plugs on each agar well. The Multiwells™ were then incubated at 22°C in the dark. After 7 days of incubation, the colony diameters of *O. piliferum*

TABLE 1. Effect of *S. similis* culture filtrates on the in vitro mycelial growth of *O. piliferum*

Incubation period (days)	Mycelial dry wt. of *O. piliferum* (mg)					
	Control			Culture filtrate of *S. similis*		
	ME	PDB	CE	ME	PDB	CE
7	19.0*d*	12.5*c*	12.0*c*	9.0*b*	5.5*a*	4.5*a*
14	27.0*d*	15.0*c*	13.5*c*	10.0*b*	6.5*a*	5.0*a*
21	35.5*e*	25.0*d*	20.5*c*	10.5*b*	7.0*a*	6.0*a*
28	42.0*e*	32.5*d*	25.0*c*	10.5*b*	7.5*a*	6.0*a*

NOTE: Values are the means of 20 replicates. Means followed by the same letter in each row are not significantly ($P = 0.05$) different from each other by Scheffé's test. ME, malt extract; PDB, potato dextrose broth; CE, carrot extract.

were measured. Sodium salts of these naturally occurring acids were prepared and their activity was also tested. Metabolites of *S. similis* were also tested against *S. similis* using the above method.

Data were subjected to analysis of variance. The individual means were compared using Scheffé's test for multiple comparison using SAS software (SAS Institute Inc. 1985).

Results

Antagonism of S. similis *and* O. piliferum

The growth of *O. piliferum* was significantly inhibited by *S. similis* at a distance when grown in dual culture on all three culture media tested (Fig. 1). The highest growth reduction of *O. piliferum* was observed on MEA.

Effect of the culture filtrate of S. similis *on the in vitro growth of* O. piliferum

The mycelial growth of *O. piliferum* was significantly reduced in liquid culture when treated with the culture filtrate of *S. similis* (Table 1). The maximum reduction of growth was observed 4 weeks after inoculation. Similarly, the mycelial growth of *O. piliferum* was inhibited when treated with the culture filtrate of *S. similis* in agar diffusion plates (Fig. 2), with mycelia of *O. piliferum* being killed when it came into contact with the culture filtrate of *S. similis*.

Interactions of S. similis *and* O. piliferum *on* P. tremuloides *wood chips*

Ophiostoma piliferum rapidly colonized *P. tremuloides* wood chips, and its darkly pigmented mycelium stained these chips black (Fig. 3*c*). *Sporormiella similis* did not cause any stain or decay in wood chips (Fig. 3*b*). When wood chips were preinoculated with *S. similis*, *O. piliferum* failed to colonize and did not stain the chips (Fig. 3*d*).

Isolation of metabolites from the culture filtrate of S. similis

Ten compounds were identified from the culture filtrates of *S. similis* by means of GC and silica gel chromatography. These were 2-methylbutanoic acid, isovaleric acid, isobutyric acid, 3,3-dimethylacrylic acid, tiglic acid, maltol, phenylacetic acid, 2-phenylethanol, *p*-hydroxybenzoic acid, and anthranilic acid (Table 2).

Effect of compounds produced by S. similis *on the in vitro growth of* O. piliferum

At 1 mg/mL, tiglic acid and 3,3-dimethylacrylic acid significantly inhibited the growth of *O. piliferum* (Table 2). Anthranilic acid, 2-phenylethanol, *p*-hydroxybenzoic acid, phenylacetic acid, isobutyric acid, isovaleric acid, 2-methylbutanoic acid, tiglic acid, and 3,3-dimethylacrylic acid significantly inhibited the growth of *O. piliferum* at 10 µg/mL, whereas sodium isobutyrate, sodium 2-methylbutyrate, sodium isovalerate, sodium phenylacetate, and maltol had no effect. At 100 µg/mL, sodium isobutyrate, sodium isovalerate, sodium phenylacetate, and maltol had no effect, whereas the remaining 10 compounds significantly inhibited the growth of *O. piliferum*. Except for maltol, all compounds significantly inhibited the growth of *O. piliferum* at 1000 µg/mL. The in vitro growth of *S. similis* was not inhibited by any of these compounds at 1 to 1000 µg/mL.

TABLE 2. Effect of compounds produced by *S. similis* on the in vitro growth of *O. piliferum*

Compound	Reduction of growth of *O. piliferum* from control (%)			
	1 µg/mL	10 µg/mL	100 µg/mL	1000 µg/mL
2-Methylbutanoic acid	0.0*a*	17.5*b*	46.5*c*	100.0*d*
Isovaleric acid	0.0*a*	42.5*b*	100.0*c*	100.0*c*
Isobutyric acid	0.0*a*	18.7*b*	100.0*c*	100.0*c*
Maltol	0.0*a*	0.0*a*	0.0*a*	0.0*a*
Sodium phenylacetate	0.0*a*	0.0*a*	0.0*a*	100.0*b*
Sodium isovalerate	0.0*a*	0.0*a*	0.0*a*	100.0*b*
Sodium 2-methylbutyrate	0.0*a*	0.0*a*	42.5*b*	100.0*c*
Sodium isobutyrate	0.0*a*	0.0*a*	0.0*a*	100.0*b*
Phenylacetic acid	0.0*a*	11.3*b*	46.5*c*	100.0*d*
p-Hydroxybenzoic acid	0.0*a*	18.7*b*	100.0*c*	100.0*c*
2-Phenylethanol	0.0*a*	25.0*b*	42.5*c*	100.0*d*
Anthranilic acid	0.0*a*	11.3*b*	100.0*c*	100.0*c*
Tiglic acid	25.0*b*	75.0*c*	100.0*c*	100.0*c*
3,3-Dimethylacrylic acid	50.0*c*	100.0*d*	100.0*c*	100.0*c*

NOTE: Data were analyzed by one-way ANOVA and Scheffé's test for multiple comparison after arcsine transformation (Zar 1984). Means followed by the same letter in each row are not significantly ($P = 0.05$) different.

Discussion

The use of fungi for biological control of plant pathogens is broadly based on their ability to compete successfully with pathogens, to be hyperparasitic, or to produce antibiotic compounds (Baker and Cook 1982; Adams 1990). *Sporormiella similis* used in this study was able to prevent stain caused by *O. piliferum* on *P. tremuloides* wood chips, suggesting a potential for bioprotection against the blue stain.

As the hyphae of *O. piliferum* were not parasitized or penetrated by *S. similis* but rather killed when they came near *S. similis* colonies, this would suggest that *S. similis* produced antifungal metabolites, which were excreted into the culture media. The chemical analysis of the crude extract of *S. similis* yielded 10 compounds that, except for maltol, were inhibitory to *O. piliferum* in vitro at concentrations ranging from 10 to 1000 µg/mL. The effect of these compounds on *Ophiostoma* spp. and other blue-stain fungi was not previously reported in the literature. It is possible that all these compounds produced by *S. similis* act simultaneously and (or) synergistically against *O. piliferum*. When grown in dual culture with *S. similis*, the colonies of *O. piliferum* were initially inhibited and then killed long before contacting mycelia of *S. similis* between the hyphae. Therefore, the antifungal activity of *S. similis* is due exclusively to its antifungal compounds rather than any parasitic function.

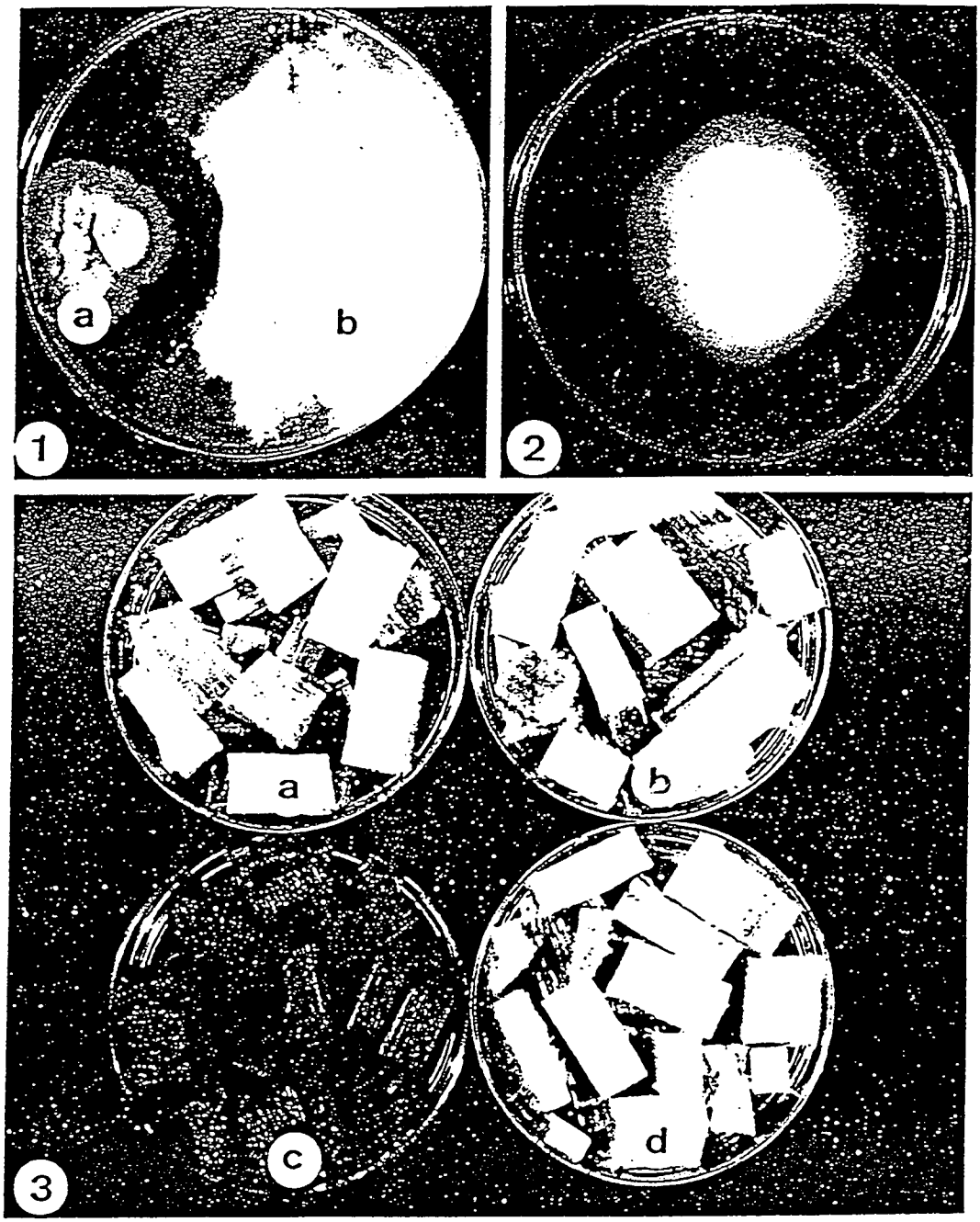

FIG. 1. Antagonism of *S. similis* and *O. piliferum* on malt extract agar medium. (*a*) *Sporormiella similis*. (*b*) *Ophiostoma piliferum*. FIG. 2. Inhibition of *O. piliferum* mycelia on agar diffusion wells when treated with a culture filtrate of *S. similis*. FIG. 3. Growth of *O. piliferum* and *S. similis* on *P. tremuloides* wood chips. (*a*) Noninoculated control. (*b*) *Sporormiella similis*. (*c*) *Ophiostoma piliferum*. (*d*) *Sporormiella similis* + *O. piliferum*.

Weber et al. (1992) isolated two compounds, similins A and B from *S. similis*, that were active against *Ascobolus furfuraceus* and *Sordaria fimicola*. Similins A and B could not be detected in our study when the fungus was grown in malt extract and soybean nutrient media. Recently, Bergstrom et al. (1993) isolated zaragozic acids from *S. intermedia*; however, their antifungal properties are not known. Weber et al. (1990) and Weber and Gloer (1991) isolated aromatic bis-ketal compounds, preussomerins A, B, C, D, E, and F from *Preussia isomera*, some of which exhibited significant antifungal and antibacterial properties. *Sporormiella similis* is an endophyte of *P. tremuloides*, which might compete successfully with *O. piliferum* for nutrition in the wood tissue.

Although *O. piliferum* does not cause any decay, the blue stain that it causes in *P. tremuloides* logs and wood chips reduces the quality of the resulting pulp and paper, especially in the chemithermomechanical pulping process. Chlorinated phenols were used in Canada for years to prevent sap stain and mould growth on unseasoned lumber and wood chips. They are no longer commercially produced in Canada because these toxic compounds persist in the

environment. Our results indicate that *S. similis* could be considered as a potential replacement candidate and serve as a biological protection agent against blue stain on *P. tremuloides*. At present it is not clear whether *S. similis* can produce inhibitory substances in the wood chips, but usually antibiotic production occurs after a period of hyphal growth has taken place, thus establishing a certain hyphal mass, age, or growth rate (Bu'Lock 1965). Changing environmental conditions on the wood chips in the form of competition from other organisms could supply impetus for the production of antifungal metabolites. For a successful infection by *S. similis* on wood chips colonized by already established *O. piliferum*, *S. similis* has to compete with *O. piliferum* in some way (i.e., production of inhibitory compounds) for limited nutrient resources.

Biological control is now being seriously considered by the forest industry for wood protection. Most bioprotection strategies have been studied mainly for their ability to prevent fungal degradation of wood products or to eliminate incipient decay; very little has been done with regard to bioprotection against wood discoloration. Recently, Zimmerman et al. (1992) stated that colorless mutant strains of *O. piliferum* are now being used on a large industrial scale as a pretreatment of wood chips to prevent staining and remove pitch before mechanical pulping. Large-scale inoculation trials of *S. similis* on *P. tremuloides* wood chips against blue-stain fungi and its effect on the pulping process are in progress.

Acknowledgement

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Appendix 6

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Antifungal activity of metabolites of Peniophora polygonia against the aspen decay fungus Phellinus tremulae

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Abstract

Ten volatile and sixteen non-volatile compounds have been isolated from culture filtrates of *Peniophora polygonia*. Among these, two volatile and three non-volatile compounds significantly inhibited in vitro growth of *Phellinus tremulae*.

Key words: Fungal metabolites – Antagonism – *Peniophora polygonia* – *Phellinus tremulae* – Volatile and non-volatile compounds.

1 Introduction

Members of the genus *Phellinus* and, in particular, *Phellinus tremulae* (Bond.) Bond. et Borisov are serious wood rotting pathogens which occur on different species of poplar (SCHRENK and SPAULDING 1909; BASHAM 1958, CERNY 1972; WIKSTRÖM 1976; WIKSTRÖM and UNESTAM 1976; HIRATSUKA 1987; HIRATSUKA et al. 1990). These fungi cause extensive damage to hardwoods in North America (BOYCE 1961). In Canada, *Ph. tremulae* seriously reduces the economic value of *Populus tremuloides* Michx. (BASHAM 1960; THOMAS et al. 1960; HIRATSUKA and LOMAN 1984; HIRATSUKA et al. 1990).

Peniophora polygonia (Pers.: Fr.) Bourdot et Galzin is the second most common cause of decay among species of *Populus*, however, it does not cause large columns of advanced decay as does *Ph. tremulae* (HIRATSUKA et al. 1990). HIRATSUKA et al. (1990) reported that many trees with decay caused by *Pe. polygonia* do not have white spongy decay caused by *Ph. tremulae*, and in trees where both fungi coexist, there are clear demarcation lines between the areas infected by each organism. NAVRATIL and WINDSHIP (1978) found that *Pe. polygonia* and *Ph. tremulae* are antagonistic to one other *in vitro*. In a recent study, CHAKRAVARTY and HIRATSUKA (1992) found that *Pe. polygonia* reduced the growth of *Ph. tremulae in vitro* when grown in paired culture on artificial media as well as on wood.

Recently AYER and TRIFONOV (1992a, b) isolated a number of compounds from *Pe. polygonia* but their effect on the *in vitro* growth of *Ph. tremulae* was not reported in detail.

In a preliminary study (unpublished), we have found that liquid cultures of *Pe. polygonia* produce ether soluble metabolites which are active against *Ph. tremulae*. The objective of this investigation was to isolate and identify the volatile and non-volatile compounds produced by *Pe. polygonia* in liquid culture and to examine their inhibitory effect on the growth of *Ph. tremulae in vitro*.

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2 Material and methods

2.1 Organisms

Peniophora polygonia (NOF 1494 = UAMH 7006) and *Phellinus tremulae* (NOF 1464 = UAMH 7005) isolated from basidiomes associated with *Populus tremuloides* were used in this study. They were maintained on carrot agar medium in Petri plates until used. These two isolates are deposited at the Northern Forestry Centre, Forestry Canada, Edmonton, Alberta, Canada and at the University of Alberta Microfungus Collection, Devon, Alberta, Canada.

2.2 Isolation and analysis of metabolites from culture filtrates of Pe. polygonia

2.2.1 V-8 Juice

Twelve 2 L Erlenmeyer flasks each containing 1 L of liquid medium [10 g glucose, 100 ml of V-8 juice (Campbell Soup Co. Ltd., Canada) filtered through Celite, and 900 ml distilled water] were inoculated with a 10 ml mycelial suspension of *Pe. polygona* (obtained by blending one V-8 juice agar colony in 150 ml of water). The liquid cultures were kept on a rotary shaker for 24 days at 23 °C. The culture broth was filtered through cheese-cloth and extracted with diethyl ether (2×2 L). The bulk of the solvent was removed under atmospheric pressure to afford a concentrated extract (5 ml) which was analyzed by gas chromatography.

2.2.2 Sabouraud medium

The Sabouraud liquid shake culture (30 g of Sabouraud dextrose broth in 1 L of distilled water) was prepared in 2 L Fernbach flasks (1 L in each flask) and incubated at 23 °C for 25 days. The culture broth was analyzed in the same manner as described above.

2.2.3 Carrot medium

The carrot liquid still culture (300 g of carrots blended in a Waring blender in 1 L of distilled water) was prepared in 2 L Fernbach flasks (1 L in each flask) and incubated at 23 °C for 15 days. The culture broth was analyzed in the same way as described above.

2.2.4 Analysis

For volatile compounds, each extract was analyzed by gas chromatography (gc), gas chromatography-infrared spectroscopy (gc-ir), and gas chromatography-mass spectroscopy (gc-ms). The identity of the compounds was confirmed using internal standards.

The volatile compounds were separated using a 5700 A Hewlett-Packard gas chromatograph equipped with a flame ionization detector. The column was an Ultra 2 crosslinked column, phenyl-methyl-silicone (5 %) 25 m × 0.32 mm id × 0.52 μ. Temperature was programmed for 60 °C (8 min), then 60–250 °C at 8 °C/min. Gc-ir analysis was performed on a 5890 Hewlett-Packard apparatus using the same column which was connected to a HP 5965 IRD system. Gc-ms spectra were recorded on a Varian Vista 600 gas chromatograph using a DB-1 column and a UG70E mass spectrometer connected with a PDP 11-83 11-250 data system.

Non-volatile compounds were separated by means of flash chromatography and preparative thin layer chromatography (Ayer and Trifonov 1992a, b).

2.3 Effect of volatile and non-volatile metabolites from Pe. polygonia on the growth of Ph. tremulae in vitro

2.3.1 Volatile compounds

For this experiment, 60 mm Petri plates containing 15 ml carrot agar medium (which was found to be the best medium for growth of *Ph. tremulae*) were inoculated singly with *Ph. tremulae* (5 mm agar plugs taken from the periphery of 2 week old mycelial mats grown in the dark) at the margin of the plate. A sterile cover slip was placed at the opposite periphery of each plate. Ten μl of each volatile compound at 1, 10, 100, and 1000 μg/ml in acetone was placed separately on the top of each cover slip. In the case of the control, 10 μl of acetone/ether (1:1) was used. Each test was replicated five times. The plates were immediately wrapped with parafilm (to trap evolving volatile compounds) and incubated at 22°C in the dark. After 3 weeks of incubation, the colony diameter of *Ph. tremulae* was measured and mycelia were observed under a microscope.

Similarly, all these volatile compounds were also tested against *Pe. polygonia* using the above method.

2.3.1 Non-volatile compounds

To test the effect of non-volatile compounds on the growth of *Ph. tremulae in vitro*, the fungus was grown on Multiwell™ Tissue culture Plates (1.8 cm × 1.5 cm, diameter × length, individual well; manufactured by Becton Dickinson Labware, NJ, U.S.A.). Two ml of carrot agar was poured into each well. Twenty five μl of each non-volatile compound at 1, 10, 100, and 1000 μg/ml in acetone was added separately to the surface of the agar in each well. In the control, 25 μl acetone was used. All the Multiwells™ were kept in a laminar flow hood for two minutes to allow the acetone to evaporate. Each agar well was individually inoculated with 5 mm agar plugs of *Ph. tremulae*, the multiwells were wrapped with parafilm and incubated at 22°C in the dark. After 3 weeks of incubation, the colony diameter of *Ph. tremulae* was measured and mycelia were observed under a microscope.

All these non-volatile compounds were also tested against *Pe. polygonia* using the above method.

Data were subjected to analysis of variance. The individual means were compared using Scheffe's test for multiple comparison using SAS software (SAS INST. INC. 1985).

3 Results

3.1 Isolation and identification of volatile and non-volatile compounds from Pe. polygonia

Ten volatile compounds were identified in the ethereal extract from culture filtrates of *Pe. polygonia* by means of gc, gc-ir, and gc-ms (Table 1). Authentic samples were used as internal standards in the gc analysis. The commercially available 2-phenylbutanoic acid (17) and 4-phenyl-3-butenoic acid (18) which are structurally related to 2-methylbutanoic acid and isovaleric acid were also tested against *Ph. tremulae*.

Sixteen non-volatile compounds were isolated from culture filtrates of *Pe. polygonia* (Table 2) by silica gel flash chromatography and preparative thin layer chromatography. Among non-volatile compounds, nine sesquiterpenes and seven aromatic compounds were reported from *Pe. polygonia* (Table 2).

fungus, or production of antifungal compounds by the antagonist fungus. Several mechanisms may act simultaneously and/or synergistically in suppressing pathogens (COOK and BAKER 1983).

In our experiment, *Pe. polygonia* produced extracellular metabolites in liquid culture which caused the inhibition of growth of *Ph. tremulae in vitro*. These metabolites may interfere with a metabolic process involving cell division, membrane function, or wall synthesis of *Ph. tremulae*. In our study, growth of *Pe. polygonia* was not inhibited by its own metabolites. This resistance of *Pe. polygonia* could be explained either by a modified permeability of its cell membrane to the metabolites or by the presence of enzymes that modify the metabolites (DEMAIN 1981).

Both volatile and non-volatile compounds isolated from *Pe. polygonia* caused significant reduction in growth of *Ph. tremulae in vitro*. Benzoic acid and 2-phenylethanol were inhibitory to *Ph. tremulae* even at 10 μg/ml. Interestingly, 2-phenylethanol has been reported to be a metabolite of *Ph. tremulae* (COLLINS and HALIM 1972). The isolation procedure, however, includes steam distillation, in which case 2-phenylethanol could be an artifact. The crude extract, the control ethereal extract, the sesquiterpene lactones 2–9 (Table 2) and the aromatic compounds 10, 13, 14, 15, and 16 (Table 2) had no inhibitory effect on the growth of *Ph. tremulae* even at 1000 μg/ml. It is possible that all the volatile and non-volatile compounds produced by *Pe. polygonia* act simultaneously and/or synergistically against *Ph. tremulae*. For ectomycorrhizal fungi, SCHISLER and LINDERMAN (1987) reported similar findings with volatile compounds in the rhizosphere of Douglas-fir.

At present it is not known whether the antifungal metabolites are produced by *Pe. polygonia* in trees previously colonized by *Ph. tremulae*. For antibiotic metabolite production to occur in the trees, some conditions for secondary metabolism would have to be met. DEMAIN (1984) stated that secondary metabolite production usually occurs after a period of hyphal growth has taken place, thus establishing a certain hyphal mass, age, or growth rate. The production can be further influenced by environmental conditions (BU' LOCK 1965). Changing conditions in the environment around a tree in the form of competiton from other organisms could supply the impetus for the production of antifungal metabolites. For a successful infection by *Pe. polygonia* in a tree colonized by already established *Ph. tremulae*, the former has to compete with the latter in some way (e. g. production of inhibitory metabolites) for limited nutrient resources.

Previous reports indicate that in *Po. tremuloides* blocks/trees, *Pe. polygonia* prevents further extension of *Ph. tremulae* and there is a clear demarcation line where the fungi came into contact with each other (HIRATSUKA et al. 1990; CHAKRAVARTY and HIRATSUKA 1992). The present work has shown that the inhibition of *in vitro* growth of *Ph. tremulae* may be due to the production of metabolites produced by *Pe. polygonia* which act simultaneously and/or synergistically against *Ph. tremulae*. Further investigation is necessary to establish whether *Pe. polygonia* has potential as a biological control agent against *Ph. tremulae* in the field.

Acknowledgements

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Summary

Phellinus tremulae is the most destructive wood rotting fungus on *Populus tremuloides*. The second most prevalent decay fungus is *Peniophora polygonia*, although it does not cause large columns of advanced decay as does *Ph. tremulae*. These two fungi are mutually exclusive or antagonistic to each other. *Pe. polygonia* produced 10 volatile and 16 non-volatile compounds in liquid culture. Among the volatile compounds, *in vitro* growth of *Ph. tremulae* was inhibited by benzoic acid at 10 μg/ml and 2-phenylethanol, 3-phenylpropanol, isobutyric acid, 2-methyl-

butanoic acid and benzaldehyde at 100 μg/ml and isovaleric acid and peniopholide at 1000 μg/ml. Two compounds closely related structurally to isovaleric acid and 2-methylbutanoic acid are 4-phenyl-3-butenoic acid and 2-phenylbutanoic acid which inhibited *in vitro* growth of *Ph. tremulae* at 1 and 100 μg/ml respectively. Among the non-volatile compounds, 5 aromatic aldehydes and 9 sesquiterpene lactones had no effect on the *in vitro* growth of *Ph. tremulae*.

Résumé

Activité antifongique des métabolites de Peniophora polygonia vis-à-vis de Phellinus tremulae, agent d'altération du tremble

Phellinus tremulae est l'agent lignivore le plus important sur *Populus tremuloïdes*; le second est le *Pheniophora polygonia* bien qu'il n'occasionne pas de colonnes d'altération aussi importantes. Ces deux champignons s'excluent l'un l'autre ou sont antagonistes. *P. polygonia* produit en culture liquide 10 composés volatiles et 16 non volatiles. La croissance *in vitro* de *P. tremulae* est inhibée par les composés volatiles suivants: l'acide benzoïque (10 μg ml^{-1}), les acides 2-phényléthanol, 3-phénylpropanol, isobutyrique, 2-méthylbutanoïque et le benzaldehyde (100 μg ml^{-1}), l'acide isovalérique, le peniopholide (1000 μg ml^{-1}). Les deux composés très proches structuralement de l'acide isovalérique et de l'acide 2-méthylbutanoïque que sont l'acide 4-phényl-3-butenoïque et l'acide 2-phénylbutanoïque sont inhibiteurs à 1 et 100 μg ml^{-1} respectivement. Parmi les composés non volatiles, 5 aldéhydes aromatiques et 9 lactones sesquiterpène n'ont aucun effet.

Zusammenfassung

Antibiotische Aktivität von Peniophora polygonia–Metaboliten gegen den Aspenfäule-Erreger Phellinus tremulae

Phellinus tremulae ist der destruktivste Fäuleerreger an *Populus tremuloides*. Der zweithäufigste Fäuleerreger ist *Peniophora polygonia*, welcher jedoch weniger ausgedehnte Fäule-Volumina verursacht als *Ph. tremulae*. Diese zwei Pilze verhalten sich antagonistisch und schließen sich gegenseitig vom Substrat aus. *P. polygonia* bildete in Flüssigkulturen 10 flüchtige und 16 nichtflüchtige Substanzen. Von den flüchtigen Verbindungen wurde *Ph. tremulae* durch Benzoesäure bei einer Konzentration von 10 μg/ml gehemmt, durch 2-Phenyläthanol, 3-Phenylpropanol, Isobuttersäure, 2-Methylbutansäure und Benzaldehyd bei 100 μg/ml sowie durch Isovaleriansäure und Peniopholid bei 1000 μg/ml. Zwei strukturell der Isovaleriansäure und der 2-Methylbutansäure sehr ähnliche Verbindungen sind die 4-Phenyl-3-Butensäure und die 2-Phenylbutansäure, welche das Wachstum von *Ph. tremulae* in vitro bei 1 bzw. 100 μg/ml hemmten. Von den nicht flüchtigen Substanzen hatten 5 aromatische Aldehyde und 9 Sesquiterpenlactone keinen Einfluß auf das Wachstum von *Ph. tremulae* in vitro.

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