# SURVEY FOR DETECTION OF THE EUROPEAN RACE OF *GREMMENIELLA ABIETINA* IN CANADA

by

C.E. Dorworth and H.L. Gross

GREAT LAKES FOREST RESEARCN CENTRE

SAULT STE. MARIE, ONTARIO

FILE REPORT

December 1978

BACKGROUND INFORMATION

Between 1970 and 1975, work at the Great Lakes Forest Research Centre defined three separate races of the conifer-infecting pathogen *Gremmeniella abietina* (Lagerb.) Morelet (Dorworth and Krywienczyk 1975). The Asian Race occurs only in northern Japan, according to extand available records (Yokota *et al.* 1974), and is mentioned only for completeness. The pathogen had been known for 100 years in Europe where it incited a disease typified by mature tree dieback (Dorworth 1971) unknown at the time of writing in North America. This pathogen was later separated as the European Race of *G. abietina* (Dorworth and Krywienczyk 1975). The North American Race occurs from the coast of New Brunswick to the western border of Ontario and in Alberta and British Columbia (Anon. 1975, Appendix B, Dorworth 1975). In the United States, its range extends from Maine to eastern Minnesota.

Extensive damage was reported in the states of New York and Vermont by a form of *G. abietina* that proved identical to the European Race by the only known test for race differentiation (Dorworth *et al.* 1977). Quarantines and embargos were erected under the statutes of several States to prevent movement of trees and tree parts from known infection areas. These were superseded by an emergency quarantine imposed by the United States Government and through Quarantine 3C/881.34 imposed by the Government of Canada, Plant Quarantine Division, Canada Department of Agriculture.

It could not be ascertained immediately if the European Race of *G. abietina* was present in Canada. The prominent mature tree dieback syndrome associated with damage by this pathogen (Skilling 1977) had not been reported from Canada, but the pathogen easily might have been overlooked in seedlings and young trees. In these plants, symptoms of

infection by the North American and European Races do not differ prominently. Furthermore, the European Race was recorded in nearly all countries bordering upon nearly 400 km of the Canadian-United States international border, and the threat of spread into Canada was imminent. A thorough detection survey by appropriately trained personnel was required to determine whether or not the European Race of *G. abietina* had in fact entered Canada in order that the pathogen might be eliminated or its spread controlled under the provisions of the Canadian Plant Quarantine Act. In the event the pathogen was not present in Canada, it was equally important to ascertain this fact with a maximum of confidence in order that a disease-free zone could be defined along the international boundary where sightings of dying trees would immediately arouse suspicion. Similarly, some form of detection survey is warranted throughout host ranges in Canada. This is required because the forest resource needs to be protected from all possible modes of entry. United States Forest Service personnel and foresters associated with the New York Department of Environmental Conservation traced the beginning of the problem to at least 1965; more than 10 years before the first quarantines were imposed. Thus, there has been ample time and opportunity for movement of infected plants into parts of Canada.

DISEASE DETECTION GROUPS

In general, the Canadian Forestry Service, Forest Insect and Disease Survey (FIDS), assisted by research personnel from each region, formed the basis of the European Race detection effort. Field personnel with the Plant Quarantine Division of the Canada Department of Agriculture concentrated upon examination of susceptible conifers imported before imposition of quarantines to insure that the pathogen had not been inadvertently

admitted. The provincial governments were alerted and the CFS trained key provincial personnel who transmitted the information to their appropriate staff. Several trades and industries also took pains to alert members and employees of the threat.

DISEASE DETECTION PROCEDURES

Under law, any group that located conifers infected by the European Race of *G. abietina* was obliged to inform the Plant Quarantine Division immediately. In fact, a spirit of mutual aid and transfer of information prevailed among all interested and participating groups.

Of particular use was the fact that the North American Race is rarely found south of (approximately) 44°30' north latitude in Canada; thus, any infection located to the south of that line must immediately arouse suspicion and receive priority attention. Pine plantations near the international boundary were to be examined in 1977 and 1978.

Methods of survey differed somewhat among the units involved across Canada. The detection survey in Ontario entailed examination of all the trees that occurred along a route that traversed two sides and the diagonals of each plantation, and was to include at least 500 trees. Small plantations were to be examined in total.

All plantings suspected of harbouring infection south of latitude 44°30'N and those bearing infection accompanied by symptoms typical of European Race infection in the north were sampled. Specimens were delivered to the nearest Canadian Forestry Service or Plant Quarantine Division laboratory, placed into sterile culture and the causal pathogens recovered. If *G. abietina* was recovered, it was sent to Sault Ste. Marie for race identification by an immunological method (APPENDIX A).

DISEASE DETECTION SURVEY RESULTS

All samples of *G. abietina* tested to determine race are listed (Table 1). Some were tested prior to the current survey but are included because they form a baseline of distribution of confirmed collections of the North American Race.

Pacific Forest Research Centre - *Gremmeniella abietina* was not found in western Canada until 1975 (Dorworth 1975), where it was later termed an indigenous pathogen by persons familiar with western forest conditions (Hiratsuka and Funk 1976). Surveys by the Pacific Forest Research Centre produced collections on *Pinus ponderosa* and *P. albicaulis* as well as one *P. contorta* var. *latifolia*; the original noted western host. All isolates tested were identical with the North American Race (Table 1). Only one additional find was made in 1978 and it came from a specimen bearing symptoms typical of infection by the North American Race (Personal communication, R.S. Hunt).

Great Lakes Forest Research Centre - Distribution of the North American Race of *G. abietina* was described by Myren and Gross (1977). The 1978 survey added several locations in northwestern Ontario, where the pathogen seems to be spreading. These locations are near Dryden, Sioux Lookout and 100 km northeast of Red Lake (Wabell Lake).

Survey for the European Race began in 1977 following a visit to the New York infection area by staff of the Great Lakes Forest Research Centre. Thereafter, the Chief Pathology Technician (P.E. Buchan) spent time with Rangers assigned to southern Ontario districts to further familiarize them with the disease. Dorworth also toured extensively in the southeastern and south-central regions with the FIDS Rangers of those areas.

In 1978, each ranger examined 20 or more plantations in his district for the presence of the disease. Total plantations examined were 95 (Fig. 1). Infections which aroused suspicion (three from the Parry Sound area) were tested for race identification in Sault Ste. Marie (Table 1). All isolates of *G. abietina* recovered were of the North American Race.

Laurentian Forest Research Centre - The general situation with respect to the North American Race of *G. abietina* in Quebec is described in the 1975 FIDS Annual Report (Anon. 1975) and records for the preceeding 10 years exist. Detection surveys for the European Race began in 1977 covering the general area to the north of New York and Vermont and up to the St. Lawrence River. In 1978, the Disease Survey Officer, D. Lachance, and several technicians spent considerable time near the New York border and located a suspect infestation in a small plantation of red pines near Power's Court, Quebec, about 4 km north of a confirmed infection by the European Race at Chateaugay, New York. Samples were removed from the infected trees, the pathogen was recovered at the Canadian Forestry Centre in Ste. Foy, Quebec, and three isolates were sent to Sault Ste. Marie where they were identified as European Race *G. abietina* (Table 1).

The Laurentian Forest Research Centre is developing, in cooperation with the Provincial Department of Lands and Forests, a listing of the location of all plantations for the nine Administrative Districts of Quebec. Plantations to the north of infection zones in the United States are to be intensively surveyed in 1979.

Maritimes Forest Research Centre - An intensive survey of New Brunswick was designed by L.P. Magasi of the Maritimes Centre and implemented by FIDS personnel and New Brunswick Provincial forestry personnel. The

Interim Report (APPENDIX B) noted that nearly one-third of the 297 plantations surveyed in New Brunswick were infected by *G. abietina*. The majority of the 97 infected plantations are in the northern one-half of the Province. Mature tree dieback, characteristic of the disease caused by the European Race, was not observed, nor did any isolates tested in Sault Ste. Marie from New Brunswick differ from the North American Race (Table 1). Percentage of plantations infected varied from 61% in the north (Bathurst Co.) to 0% in the south (St. John Co.).

An actual reduction in the number of plantations infected in Nova Scotia was recorded according to Magasi. This may indicate attrition of imported North American Race infections within an area to which they could not adapt.

CONCLUSION

Conversation with personnel of the Plant Quarantine Division in November, 1978, revealed the intention of the Division to declare the infected plantation in Quebec under "Quarantine Detention". No products could be moved from the plantation until it was sanitized to free it of *G. abietina*. Because the plantation is near infected plantations in the state of New York, annual inspection would be required. Plant Quarantine will work closely with the Laurentian Forest Research Centre and the Province of Quebec, Department of Lands and Forests, to establish procedures to identify other potential infection sites in the same general area.

The Quebec plantation infection is not very important in terms of geographic spread of *G. abietina*. It lies at the northern edge of an intermittent wooded area contiguous with that in New York, but to the south of a broad belt of predominantly agricultural land in southern Quebec. The

next jump, into the northern forested areas, is the one of consequence, as this would extend the infection area into the major northern forest zone of Canada. However, further incursion should proceed with difficulty unless small plantations and ornamental conifers serve as "stepping-stones" across the agricultural belt, or the pathogen is carried across by hand. The term "should proceed" reflects our lack of knowledge regarding effective long-distance spread of the pathogen in the field.

Politically, the infection is important as it represents a confirmed European Race infection within Canada, and could be used as the basis for retaliatory quarantines. Administratively, the infection is important as the protocol developed in this case will be cited as a precedent in future situations of the same type. There is little chance of avoiding further small infections of this type, as the European Race occurs within 60 miles of the international border over a distance of *ca.* 400 km, and comes to within 3 km of the border at Power's Court, Quebec.

The history of the disease in Europe and, with a far less substantive basis, in New York indicates it responds strongly to weather conditions. The European Race may be effectively "dormant" or endemic in an area over one or more dry years only to become epidemic when one or more cool, moist years prevail. It is often overlooked when endemic, and areas erroneously could be declared free of the European Race. Any rigid formula for erradication and control is bound to fail one time or another unless total destruction of host plants is involved. Evidently this will not be practical in some situations. The Power's Court situation is one in which erradication of the pathogen without loss of the plantation is at least possible and failure would probably result in loss of a single plantation.

This may not be the case in an area of contiguous or close intermittent plantings of susceptible conifers. If the disease becomes prevalent in Canada, foresters will have the opportunity to observe it in all its forms. There would be certain advantage to excluding it from Canada so long as possible while gaining knowledge from observations in the United States.

LITERATURE CITED

Anon. 1975. Forest Insect and Disease Survey annual report 1975. Can. For. Serv., Ottawa, 103 p.

Dorworth, C.E. 1971. Diseases of conifers incited by *Scleroderris lagerbergii* Gremmen: A review and analysis. Can. For. Serv. Dept. Pub. 1289, 42 p.

Dorworth, C.E. 1975. *Gremmeniella abietina* collected in Alberta, Canada. Plant Dis. Reporter 59:272-273.

Dorworth, C.E. and J. Krywienczyk. 1975. Comparisons among isolates of *Gremmeniella abietina* by means of growth rate, conidia measurement, and immunogenic reaction. Can. J. Botany 53:2506-2525.

Dorworth, C.E., J. Krywienczyk and D.D. Skilling. 1977. New York isolates of *Gremmeniella abietina* (*Scleroderris lagerbergii*) identical in immunogenic reaction to European isolates. Plant Dis. Reporter 61:887-890.

Hiratsuka, Y. and A. Funk. 1976. Additional records of *Gremmeniella abietina* in Western Canada. Plant Dis. Reporter 60:631.

Myren, D.T. and H.L. Gross. 1977. Distribution of orgasams causing important forest tree diseases in Ontario. Can. For. Serv. Info. Rept. O-X-262, 136 p.

Skilling, D.D. 1977. The development of a more virulent strain of *Scleroderris lagerbergii* in New York State. Europ. J. For. Pathology 7:297-302.

Yokota, S., T. Uozumi and S. Matsuzaki. 1974. Scleroderris canker of Todo-fir in Hokkaido, northern Japan. I. Present status of damage, and features of infected plantations. Europ. J. For. Pathology 4:65-74.

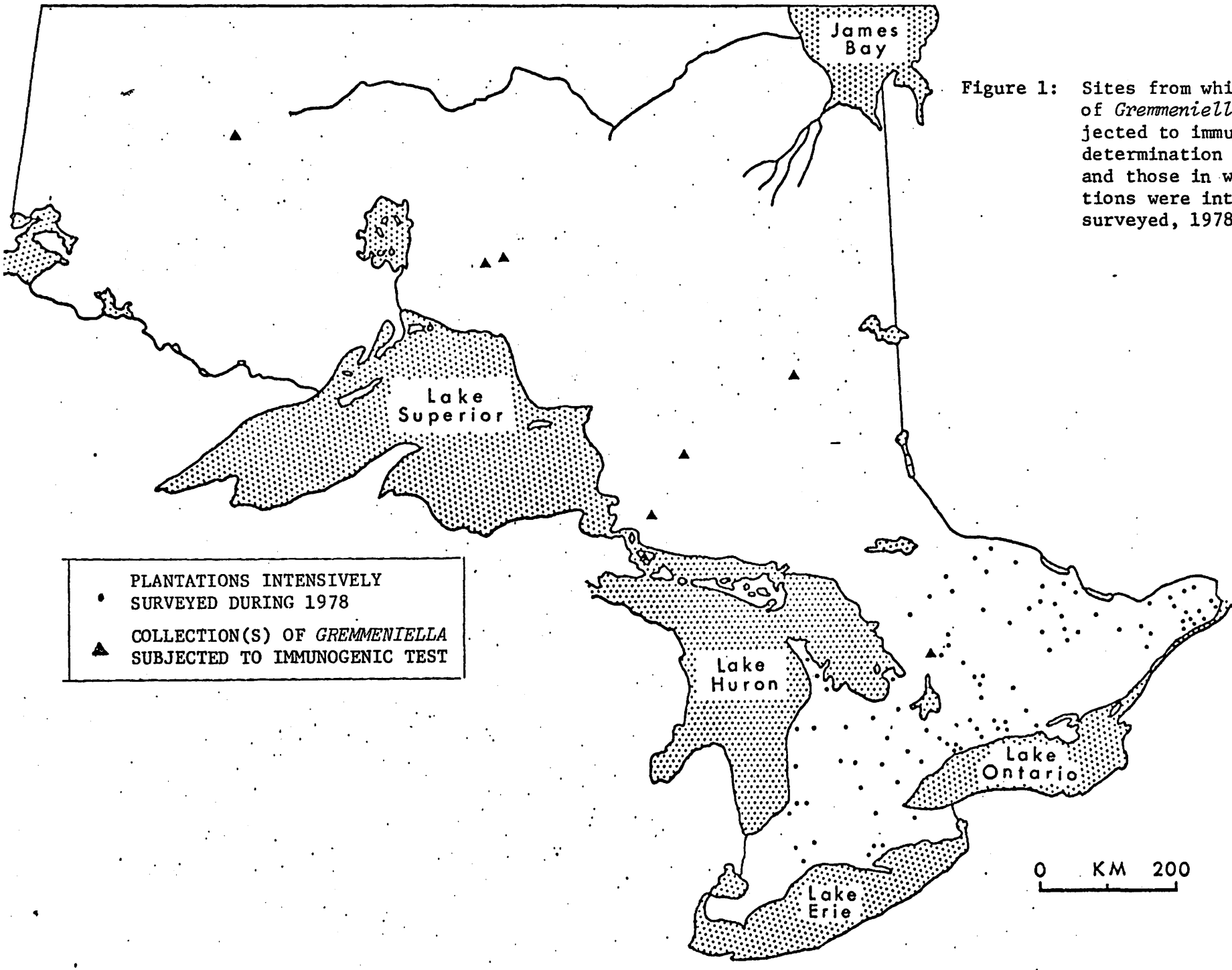



Figure 1: Sites from which collections of *Gremmeniella abietina* subjected to immunogenic race determination were obtained and those in which plantations were intensively surveyed, 1978.

Table 1. Origin and description of isolates of *Gremmeniella abietina* tested to determine race.

| No. of Isolates | Place of Collection | Agency Submitting | Number of Isolates | |
|---|---|---|---|---|
| | | | European | North American |
| 4 | Alberta<br>British Columbia | PFRC | | 4 |
| 17 | Ontario | GLFRC | | 17 |
| 5 | Quebec | LFRC | 3 | 2 |
| 3 | New Brunswick | MFRC | | 3 |
| 13 | Michigan<br>Wisconsin<br>New York<br>Vermont | USFS | 6 | 7 |
| 1 | Vermont | Univ. Vt. | 1 | |
| 1 | New York | N.Y. | | 1 |

Agency:
- PFRC - Pacific Forest Research Centre
- GLFRC - Great Lakes Forest Research Centre
- LFRC - Laurentian Forest Research Centre
- MFRC - Maritimes Forest Research Centre
- USFS - United States Forest Service<br>North Central Forest Experiment Station
- Univ. Vt. - University of Vermont
- N.Y. - New York Department of Environmental Conservation

# APPENDIX A

C. E. Dorworth
November 1977

# A. GREMMENIELLA ABIETINA - ANTISERUM PRODUCTION

1. Obtain New Zealand white rabbits, 6 - 12 months, ca. 5 kg. Retain ca. 2 weeks to insure they are in good condition and to permit acclimatization.

2. Prepare emulsion of fungus antigens (soluble protein solution) fraction and Freund complete bacto adjuvant;

   0.4 ml antigens
   0.6 ml phosphate buffer
   1.0 ml adjuvant

   *ALTERNATIVE
   0.9% NaCl

   Emulsion phase is achieved by mixing above ingredients in 5 ml beakers with a Virtis 'Precise' homogenizer at setting 30, equipped with a micro shaft and micro blades, for one minute. The mix becomes thick and milky once the aqueous and oil phases are emulsified

   1) Means of achieving emulsion phase is unimportant but prevent foaming with consequent introduction of gaseous phase (air).
   2) I have no experience with Incomplete Freund adjuvant
   3) Phosphate buffer listed above is used for first injection, then replaced with antigen prep in second and third injections (below). Phosphate buffer may be replaced in first injection as well if relatively weak protein solutions were recovered from fungus.
   4) Protein conc. was not determined prior to injection but rather tissue extracts were dialyzed to point of precipitation before use.

3. Inoculate rabbits with above. Recovery from 2 ml ingredients $\simeq$ 1.2 ml emulsion.

   1) First injection: dorsal intradermal; rest 1 week
   2) Second injection: intraperitonial; rest one week
   3) Third injection dorsal intradermal; rest 10 days
   4) Three intraveneous (marg. ear) injections follow at 3 day intervals, increasing dosage of (nonemulsified) antigen prep from 0.3 to 0.6 to 1.0 ml resp. Rest one week.
   5) Bleed to yield 5 to 10 mls., separate serum over 1 - 2 hours, centrifuge to eliminate debris and test titer. If 1:8 dilution yields adequate reaction, bleed rabbit to recover total serum, separated as above, and freeze in closed ampules. Alternatively, if titer is low, a second set of 3 to 5 injections may be added, including one or two intradermal and another set of intraveneous, a second determination is made to determine titer and the rabbit is either bled by heart puncture if titer is adequate or discarded and the procedure repeated with a second animal. Alternatively, low titer serum may be concentrated with some success (method attached).

C. E. Dorworth
November 1977

B. GREMMENIELLA ABIETINA - CULTIVATION OF FUNGUS

1. Filter Campbell's V-8 juice first through cheesecloth, then through coarse filter paper and finally through Whatman's No. 1 paper or equivalent. Freezing the filtered juice, thawing and then refiltering through fine paper further clarifies the juice.

2. Growth medium is comprised of 2.5 g dextrose:25 ml filtered V-8 juice:225 ml distilled water/500 ml Erlenmeyer flask. Flasks are plugged with foam plugs capped with aluminum foil and autoclaved 20 min., after which they might be stored for 1 or 2 months. Aluminum caps are discarded after inoculation of medium

3. Cultures to be tested are grown on V-8 agar plates. Flasks are aseptically inoculated with bits of mycelium from the plate cultures (or spores if these appear) with an inoculating needle. Flasks are incubated at 20°C for 30 days. The only agitation is supplied when the flasks are swirled every second day to submerge mycelium that appears on the surface. This avoids mycelial clots and surface growth, both of which are difficult to comminute.

4. After 30 days, filter away the growth medium with a buchner funnel (vacuum), wash the mycelial mat with 500 ml of distilled water and filter that away until the mat is moist (but never dry). This mat is best extracted now, but has been retained frozen in a sealed container (taped Petri dish) for up to a month and good results still obtained.

5. Additional: The procedure is, for purposes of these tests, nonquantitative. We use 10 to 15 flasks to yield final protein (antigen) solution of 10 to 20 ml of 1000 ppm $N_2$. This is best for general use but to identify single isolates in fair numbers, possibly 2 or 3 flasks would be sufficient. If everything worked perfectly every time, 1.0 ml of extract to use in $N_2$ determination plus 0.1 ml for immunodiffusion tests would be adequate.

C. E. Dorworth
November 1977

## C. GREMMENIELLA ABIETINA - SOLUBLE PROTEIN EXTRACTION

1. Soluble proteins of G. abietina are the product used both in generation of immune serum and in later immunodiffusion tests, and will be termed also, the "antigen solution" or "preparation".

2. A number of means of comminuting the mycelium are available. In Sault Ste. Marie, we used:

   a). Mortar & Pestle-The mortar and pestle plus acid-washed sand, obtainable through the chemical companies, are stored in the freezer. Enough sand to cover the mycelial mat is added into the mortar thereafter and the mix ground with additions of phosphate buffer as required to insure proper grinding and establishment of a liquid phase.

   | | | |
   |---|---|---|
   | Buffer= | $KH_2PO_4$ | 13.61 g/l |
   | | $Na_2HPO_4$ | 26.81 g/l |
   | | NaCl | 34.0 g/l |

   The entire business is nonquantitative at this point and done by eye and by experience. Once grinding is complete, and examination of a bit of the mess with a microscope will tell if intact cells remain, the liquid may be drained away and the sand washed several times with buffer. Again, there are no rules to quantities. I use colour of extract to give an idea when most of the soluble materials are recovered, though pigments are probably not involved in immune reactions. The more liquid is recovered, the more there will be to concentrate. Experience will provide a compromise. Keep the mix as cool as possible.

   b) Braun Homogenizer- Use about twice the "volume" of 1.00 to 1.05 mm glass beads as fungus mycelium, and add phosphate buffer to the top of the mycelium-bead layer in the Braun flask. Again, this is merely a suggestion which can be altered with experience. Cool the flask for ca. 10 seconds and operate the homogenizer for another one minute. Check the grind with a microscope and grind further as required. Usually one minute is sufficient but some isolates yield resistant clods of mycelium. Do not run a great enough volume of $CO_2$ to freeze flask contents. Once grinding is completed, filter away beads and particulates. Regardless of method, either centrifugation or millipore filtration is required to yield a good, clean prep.

3. The raw filtered mycelial extract should be kept cooled (above freezing) as much of the time as possible. Place extract in a tube of dialysis membrane of convenient size and place in a container of Carbowax PEG 4000 at 2°C until or preferably just before solids begin to precipitate from the antigen mix. Transfer the liquid from the tube to a glass container, through a fine filter if precipitation has occurred, and remove one ml for $N_2$ determination. Keep the remainder just above freezing until time for immunodiffusion tests. If such tests are to be delayed several weeks, freeze the antigen mix in sealed containers. Freeze and thaw as infrequently as possible as this lessens the life of the antigen prep. Suggest the prep be divided into several lots of convenient size such that one thawing is enough for any lot, after which it is discarded (the unused portion).

4. Method of $N_2$ determination is not necessarily dictated by test requirement but by convenience. We use micro Kjeldahl tests as these are available, but double-diffusion and spectraphotometric determinations are used with equal success elsewhere. Use one method though as results are not necessarily consistent among tests where different methods are employed.

5. Adjust all samples to be tested in a lot, to the same $N_2$ concentration. Concentration of 1000 ppm $N_2$ is routinely used in this laboratory with good success, but that is not a rule. Here, as in many places, either physiologic saline or phosphate buffer may be used to dilute to 1000 ppm or whatever. Once this step is completed the test becomes quantitative. Earlier points such as be guided by convenience in adding indefinite quantities of this and that, no longer apply. It is quite important that the concentration of 1000 ppm or whatever be maintained in order that results within a single immunodiffusion test will reflect differences in isoltes rather than differences in test conditions.

C. E. Dorworth
November 1977

D. GREMMENIELLA ABIETINA - IMMUNODIFFUSION

1. An 8 cm x 10 cm x 0.1 cm Kodak glass cover plate serves as a useful reaction plate in these reactions as it will bear 6 sets of immune reactions (24 unknowns), has minimal surface irregularities and good optical qualities, and is of a convenient size to store. Smaller plates are also useful, depending upon requirements.

   If I had a large number of routine tests to perform, I would at least look into use of 5 cm Petri plates. Optical properties and surface irregularities are unsatisfactory for many uses but, if they give the answer, a great deal of time would be saved over a number of tests.

   Regardless, acid wash the glassware.

2. Cover the reaction surface (of the slide, Petri dish) with a 1 to 2 mm thick layer of 1% agar. I use 15 ml per 8 x 10 cm plate. Permit to dry entirely.

3. Cover the dry agar layer with a solution of 1% agarose (Seakem, Bausch & Lomb - - others are available but this this is dependably good). The agarose may be melted in a closed beaker (aluminum foil over) at 2% in a boiling water bath and mixed thereafter with an equal quantity of 1.8% NaCl in water to yield 1% agarose in physiologic saline solution. This is applied to the slide with a large volume pipette to a depth of 2 mm (approximately). I use 18 ml/8 cm x 10 cm plate. This is the time-consuming part that might be eliminated by use of small Petri dishes. Also, the dish, once sealed with plastic tape, is its own moist chamber. Enough about dishes. Use a level surface to prevent tilting of the layer with resultant difference in thickness from one side of the slide to the other. Permit agar to gel and place the slide in a moist chamber, possibly in the refrigerator. Cool agar punches to yield cleaner wells than warm agar but with the disadvantage that its develops a bit of condensate at times.

4. Punch holes per some template appropriate to the work through the agarose. A cork borer may perforate the underlayer of dried agar as well and a somewhat blunted instrument is preferred, such as a glass ampule.

5. Considering only a setup employing a centre well and 5 surrounding wells (attached template); the centre well will contain antiserum and the surrounding wells the antigen preps. One surrounding well must always contain antigen complimentary to the antiserum (ie.; antigens of Sf-4 and antiserum produced in response to injections of antigens of isolate Sf-4). The others

may contain unknowns though I often place antigen prep of a known North American Race isolate in one, next to that of the known European prep, for total internal comparison. That, of course, restricts the usefulness of a single setup to 3 unknowns.

6. Use a fine-drawn disposable pipette to fill each well to the top of the agar layer. If the wells contain water after removal from the fridge, remove with a pipette to insure equal quantities of antigen are placed in each well. Similarly, if the agarose layer is found to be of unequal depth across the plate, discard.

   Work quickly enough to limit desiccation of the agarose and loss of preps but with particular attention to neat application into each well. If mixing is suspected (it was bumped, a drop sprayed.....),repeat the test. If the agarose layer is noticeably mobile on top of the glass underrun of solutions may occur with subsequent mixings and repeat will be necessary. The result will, at least be obvious upon staining.

   Finally, place the slide in a moist chamber for 24 to 48 hours at 20°C. The particular reaction of importance here is usually complete in 24 hours.

## SAMPLE TEMPLATES

C. E. Dorworth
November 1977

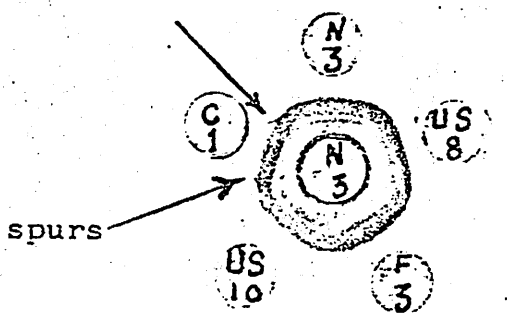
## E. GREMMENIELLA ABIETINA - IMMUNODIFFUSION PLATE INTERPRETATION

1. One can spend a great many hours in immunodiffusion slide interpretation and at times the results will be contradictory and require subjective judgement. The present test, employing as it does a single distinct precipitation line to differentiate between two groups of G. abietina isolates (North American and European Races) has proved quite objective and entirely consistent to date. This only means that the exceptions are yet to be found. Nothing within the test has sacred qualities but it would be helpful if new or changed procedures or interpretations were experimentally linked with the original in order to maintain continuity. End philosophy.

2. The test layout employing wells alone is less expensive in antiserum/unknown than that employing wells and troughs. The well-trough design provides for a greater number of comparisons within a single test. Considering the well-well design, I regard a single test as one employing the requisite homologous reaction (as SF-4 x SF-4), probably an nonhomologous known reaction (as SF-4 x SC-1) plus three unknowns. This is highly conservative but insures an internal control for each test.

3. Five confluent precipitation lines are formed between the antiserum and antigen wells, irrespective of origin of G. abietina isolates employed. These may be disregarded in the present test. A sixth line is confluent between antiserum and antigen wells containing reactants of European Race and New York isolates but this line fails to form when antigens of North American Race isolates are reacted against European Race antiserum. A confluent precipitation line produced among a set of European or N.Y. isolates (as response to antigens of these in the peripheral wells) will terminate if North American Race antigens occur in one of the wells. Ends of the otherwise confluent line are sometimes referred to as "spurs". See attached figure. These identify the position of the nonhomologous reaction or of antigens of the North American Race isolate.

   For mass testing, a refinement of the technique is in order, if feasible. One might consider precipitating with the North American antigen, reactive components in the European Race antiserum. Thereby, only the reactants responsible for the single differentiating precipitation line in the homologous reaction will remain. Future tests become positive or negative in terms of a single line, deleting consideration of spurs, skewed patterns, etc. The agarose layer itself might be deleted in favour of precipitation reactions in depression plates. This is a fairly standard technique worth testing.




## TYPICAL REACTIONS

| EUROPEAN RACE | NORTH AMERICAN RACE |
|---|---|
| N-3: Norway | C-1: Ontario |
| F-3: Finnland | |
| US-8: New York | |
| US-10: New York | |

Isolate details appear in Can. J. Bot. 53:2506-2525 (1975).

#

# APPENDIX B

# SCLERODERRIS CANKER IN NEW BRUNSWICK IN 1978

(an interim report)

L. P. Magasi

Forest Insect and Disease Survey
Maritimes Forest Research Centre
Fisheries and Environment Canada
Fredericton, New Brunswick

August 1978

# SCLERODERRIS CANKER IN NEW BRUNSWICK IN 1978

## Introduction

Scleroderris canker of pines has been known in New Brunswick since 1971 (Magasi 1972). The importance of the disease has been repeatedly pointed out and the opinion recently expressed that the incidence of the disease is underestimated in the Maritimes (Magasi 1977).

A disease of seedlings and trees, it is caused by the fungus Gremmeniella abietina (Scleroderris lagerbergii). A new strain of the fungus was found in parts of the United States adjoining Canada in the mid-1970's which is capable of killing not only young pines but mature trees as well. Pole size stands may be killed within two or three years by the "new", so-called European strain. Recent studies in the United States (unpublished) show that the European strain is not confined to pines but is capable of infecting a wide range of coniferous species.

Concern over the spread of the European strain towards Canada resulted in a decision by the Canadian Forestry Service and the Plant Protection Division of Agriculture Canada to conduct surveys in pine plantations in 1978 to determine if the new strain has entered Canada. Concern over the distribution of the "old", so-called North American strain, and over the apparent lack of understanding of its potential resulted in a decision at the Maritimes Forest Research Centre to extend the survey beyond its original objective of investigating only plantations of recently imported stock to a detailed pine plantation survey. The destruction of over a million seedlings by the disease in an industrial forest nursery

was an unexpected "assist" at the beginning of the season. Consequently, the Forest Insect and Disease Survey had the cooperation of both provincial and industrial forestry concerns throughout the season.

This interim report is intended to summarize the results to date, to put the information , as it stands, into the hands of forest managers now, to help make decisions on cleaning up or destroying infected plantations, on planning reforestation programs etc., and to show that Scleroderris canker in New Brunswick is an economically important forest disease.

## Results

The disease was found in all but three counties in the Province in 1978. The disease is known to be present in one of these, (Albert County), and also at a few additional locations not surveyed this year in other counties (Magasi, 1977) (Fig. 1).

Nearly a third (32.6%) of the 297 plantations surveyed were found infected by G. abietina.

The majority of the 97 infected plantations are in the northern half of the Province. (Fig. 2).

The situation is most serious in the Central and in the Northeast Regions where more than half of the plantations surveyed were found infected (Fig. 2, Table 1).

Most of the infected plantations in the Southwest Region are in Carleton and in northern York counties (Fig. 3, Table 2).

Plantations were found infected at about the same frequency regardless of the species of pine (Fig. 4, Table 3).

Severity of infection based on the number of trees involved (with no regard to the condition of infected trees) was much higher on

jack pine than on red pine, probably due to differences in plantation age (Table 4).

A list of infected plantations in each Forest Region is attached (Table 5-9).

## Comments

Recommendations are planned for the final report, a few comments here may be appropriate.

Infected plantations should be "cleaned up", partly to control the spread of the disease in the plantation, partly to prevent the fungus from spreading into nearby plantations. Sanitation should involve destruction of infected small trees and pruning infected larger ones. Plantations with high severity rating may have to be eliminated.

Non-infected plantations should be regularly inspected in the early years after establishment for early detection and control of infection. Planting in infected areas, which encompasses much of the northern half of the province, should take the proximity of known infection points into consideration.

Nurseries should be protected in areas of infection.

Investigative work (research?) is necessary to study some of the implications of the situation (the rate of spread, mortality etc.) in the different Regions to refine control procedures.

It is obvious that Scleroderris canker is and will remain an economically important factor in the proper management of pine forests in New Brunswick.

All the above relates to the "old" North American strain of the disease - the possible implications of the European strain, if it ever reaches the Maritimes, have not been considered.

## A Request

I would appreciate comments and suggestions for the organization of the final report. What should be included, what of the above is of no interest, what other aspects should be analyzed etc. I am trying to make the report useful for the forest manager, so I would rather have your suggestions now than your criticism later.

## Literature Cited

Magasi, L. P. 1972. First record of Scleroderris lagerbergii in New Brunswick, Canada. Plant. Dis. Reptr. 56: 245-246.

Magasi, L. P. 1977. Forest Pest Conditions in the Maritimes in 1977 with an outlook for 1978. MFRC Info. Rept. M-X-82.

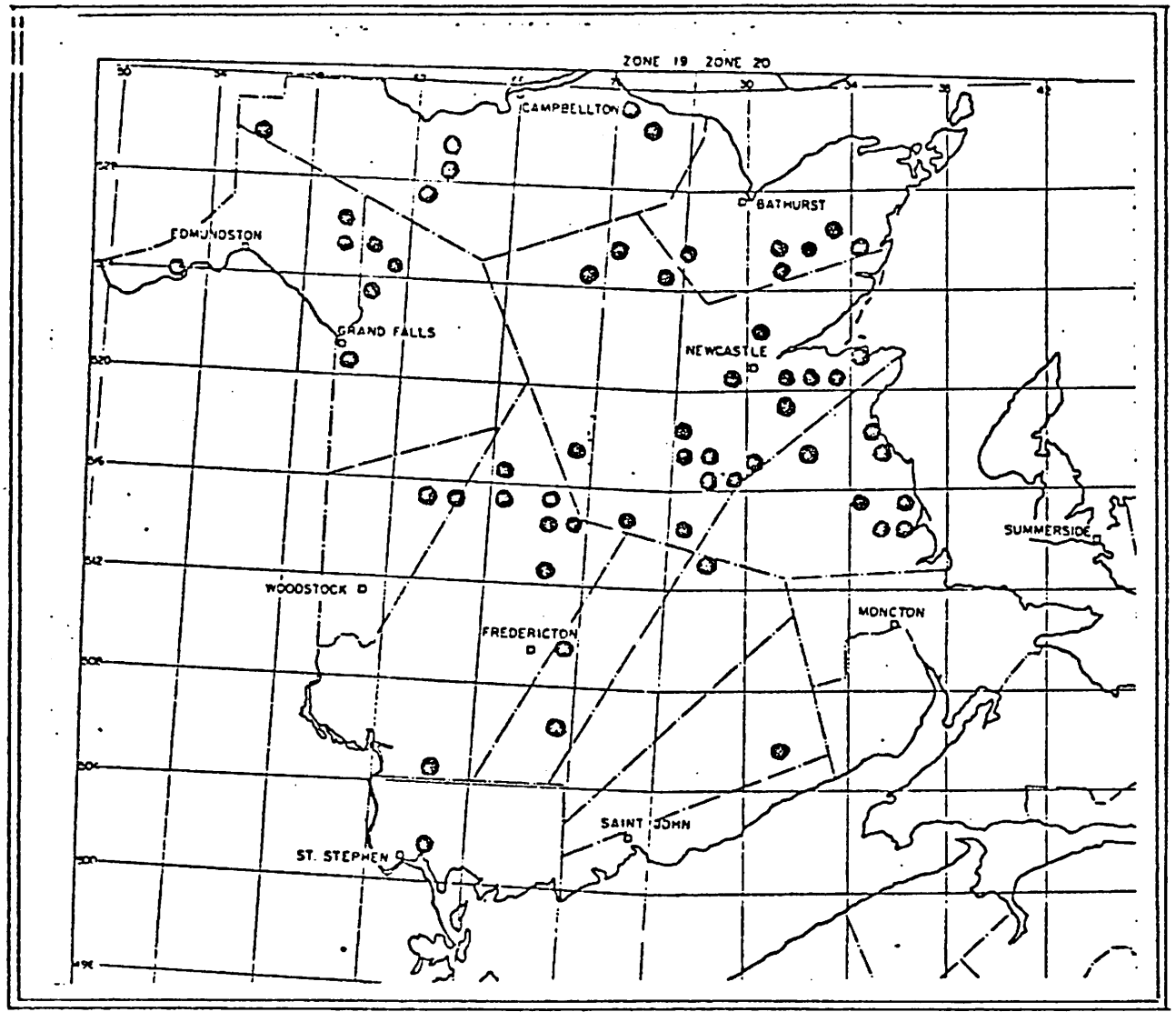



Fig. 1. Areas affected by Scleroderris canker in New Brunswick - 1978.

(Aug. 31)

Forest Insect and Disease Survey
Maritimes Forest Research Centre

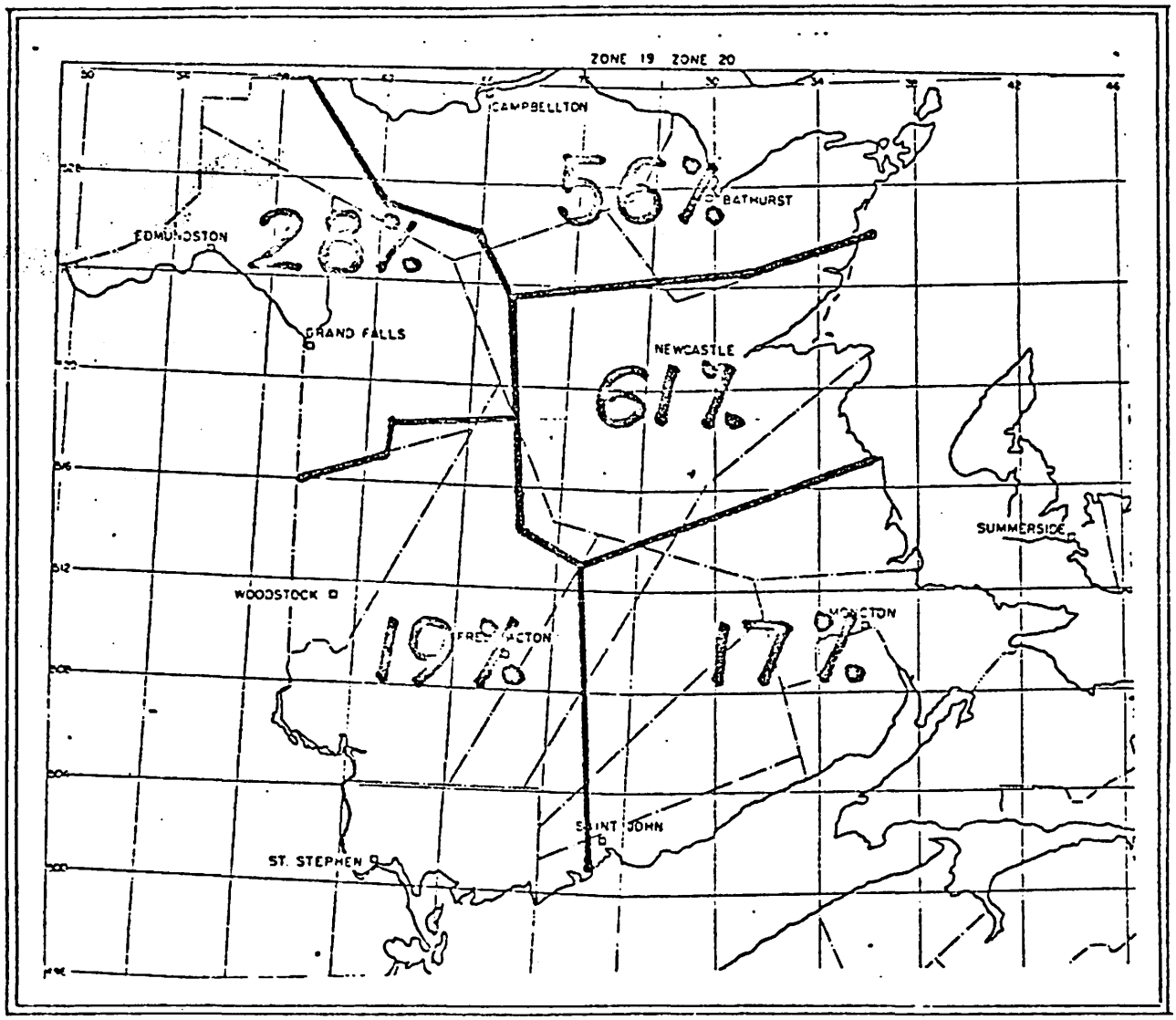



Fig. 2. Percentage of plantations affected by Scleroderris canker in the five Forest Regions in New Brunswick - 1978

(Aug. 31)

Forest Insect and Disease Survey
Maritimes Forest Research Centre

Table 1

The occurrence of Scleroderris canker in the five New Brunswick forest regions

| Region | Plantations examined | Scleroderris found | % infection |
|---|---|---|---|
| 1. Northeast | 39 | 22 | 56.4 |
| 2. Central | 56 | 34 | 60.7 |
| 3. Southeast | 65 | 11 | 16.9 |
| 4. Southwest | 91 | 17 | 18.7 |
| 5. Northwest | 46 | 13 | 28.3 |
| Province | 297 | 97 | 32.6 |

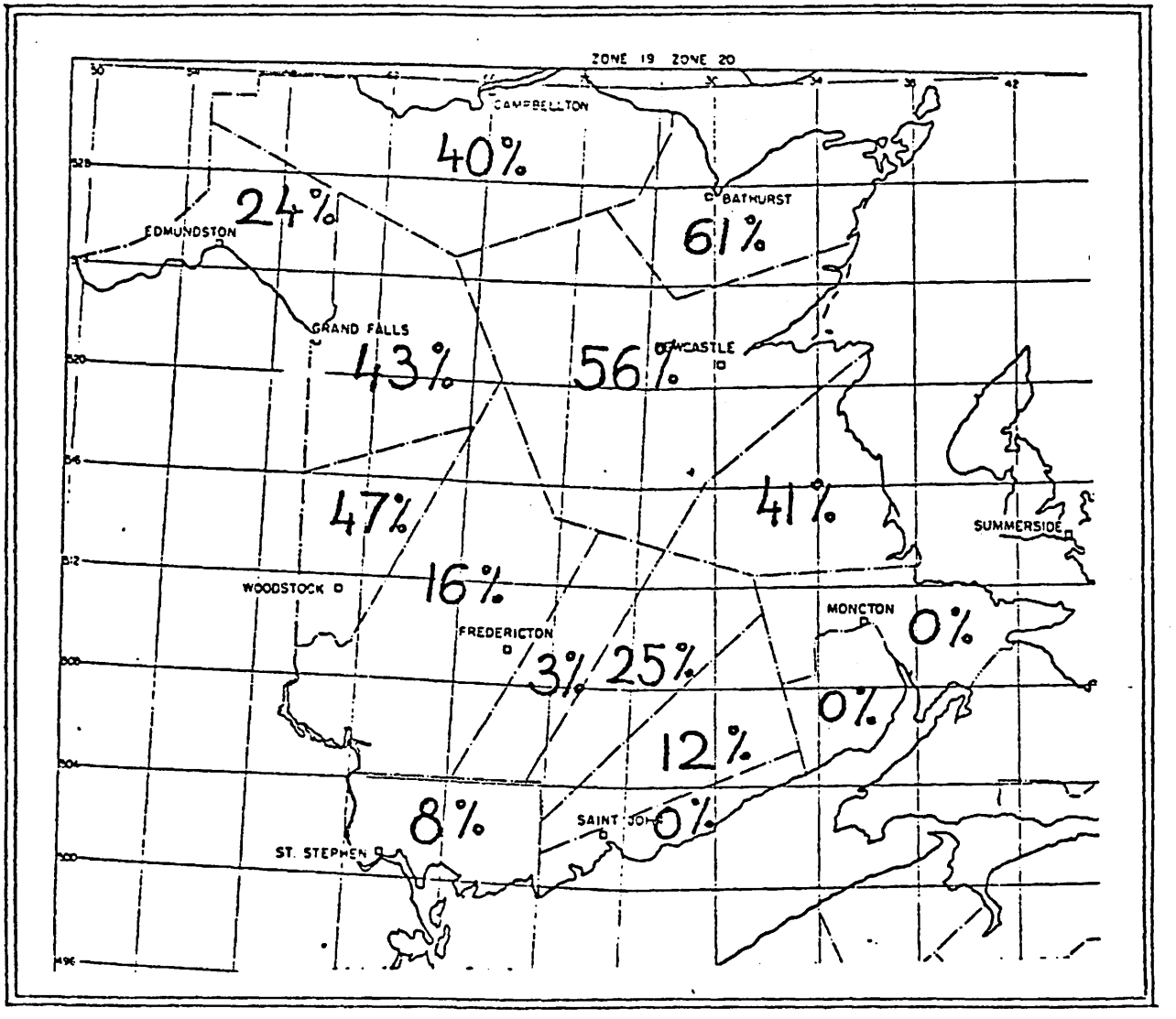



Fig. 3. Percentage of plantations in each county affected by Scleroderris canker in New Brunswick - 1978.

(Aug. 31)

Forest Insect and Disease Survey
Maritimes Forest Research Centre

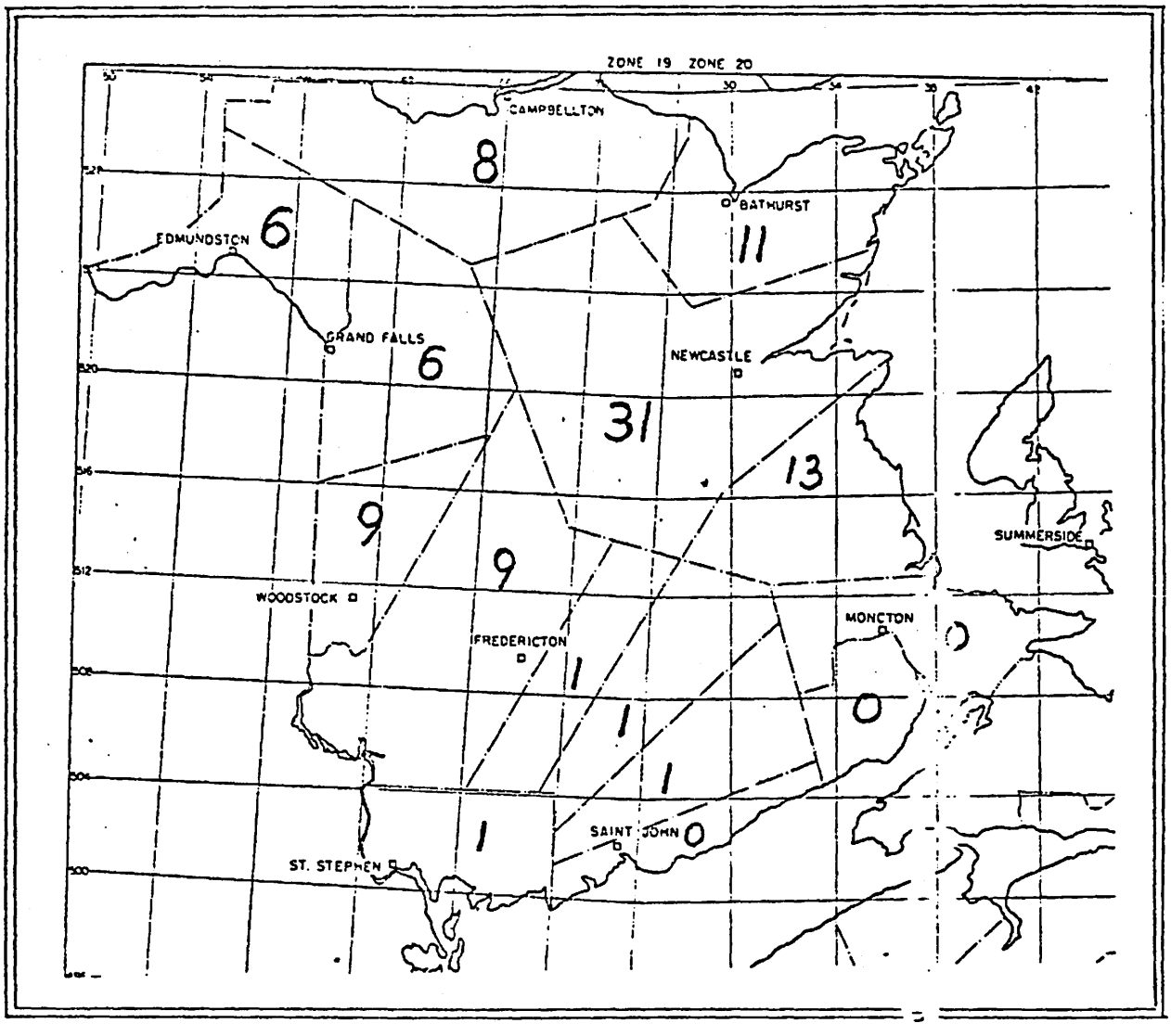



Fig.4 . Number of plantations in each county where Scleroderris canker was found in New Brunswick - 1978

(Aug. 31)

Forest Insect and Disease Survey
Maritimes Forest Research Centre

Table 2

The occurrence of Scleroderris canker by host in the different counties in New Brunswick

| County | jP | | | rP | | | scP | | | Other | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | P | % | S | P | % | S | P | % | S | P | % | S | P | % |
| Rest. | 11 | 6 | | 7 | 2 | | 1 | - | | 1 | - | | 20 | 8 | 40.0 |
| Glou. | 12 | 5 | | 6 | 6 | | - | - | | - | - | | 18 | 11 | 61.1 |
| North. | 27 | 13 | | 19 | 11 | | 9 | 7 | | - | - | | 55 | 31 | 56.4 |
| Kent | 26 | 8 | | 1 | 1 | | 4 | 3 | | 1 | 1 | | 32 | 13 | 40.6 |
| West. | - | - | | - | - | | 1 | - | | - | - | | 1 | - | - |
| Albert | 2 | - | | - | - | | - | - | | - | - | | 2 | - | - |
| St. John | - | - | | - | - | | - | - | | - | - | | - | - | - |
| Kings | 4 | 1 | | 3 | - | | 1 | - | | - | - | | 8 | 1 | 12.5 |
| Queens | 2 | 1 | | 2 | - | | - | - | | - | - | | 1 | 1 | 25.0 |
| Sun. | 18 | 1 | | 8 | - | | 2 | - | | 2 | - | | 30 | 1 | 3.3 |
| Charlotte | 2 | - | | 5 | 1 | | 1 | - | | 4 | - | | 12 | 1 | 8.3 |
| York | 25 | 1 | | 19 | 2 | | 12 | 5 | | 1 | 1 | | 57 | 9 | 15.8 |
| Carleton | 4 | 4 | | 9 | 5 | | 6 | - | | - | - | | 19 | 9 | 47.4 |
| Vict. | 7 | 3 | | 3 | 2 | | 4 | 1 | | - | - | | 14 | 6 | 42.9 |
| Mad. | 9 | 5 | | 13 | 1 | | 3 | - | | - | - | | 25 | 6 | 24.0 |
| Totals | 149 | 48 | 32.2 | 95 | 31 | 32.6 | 44 | 16 | 36.4 | 9 | 2 | 22.2 | 297 | 97 | 32.6 |

S - Surveyed

P - Scleroderris found

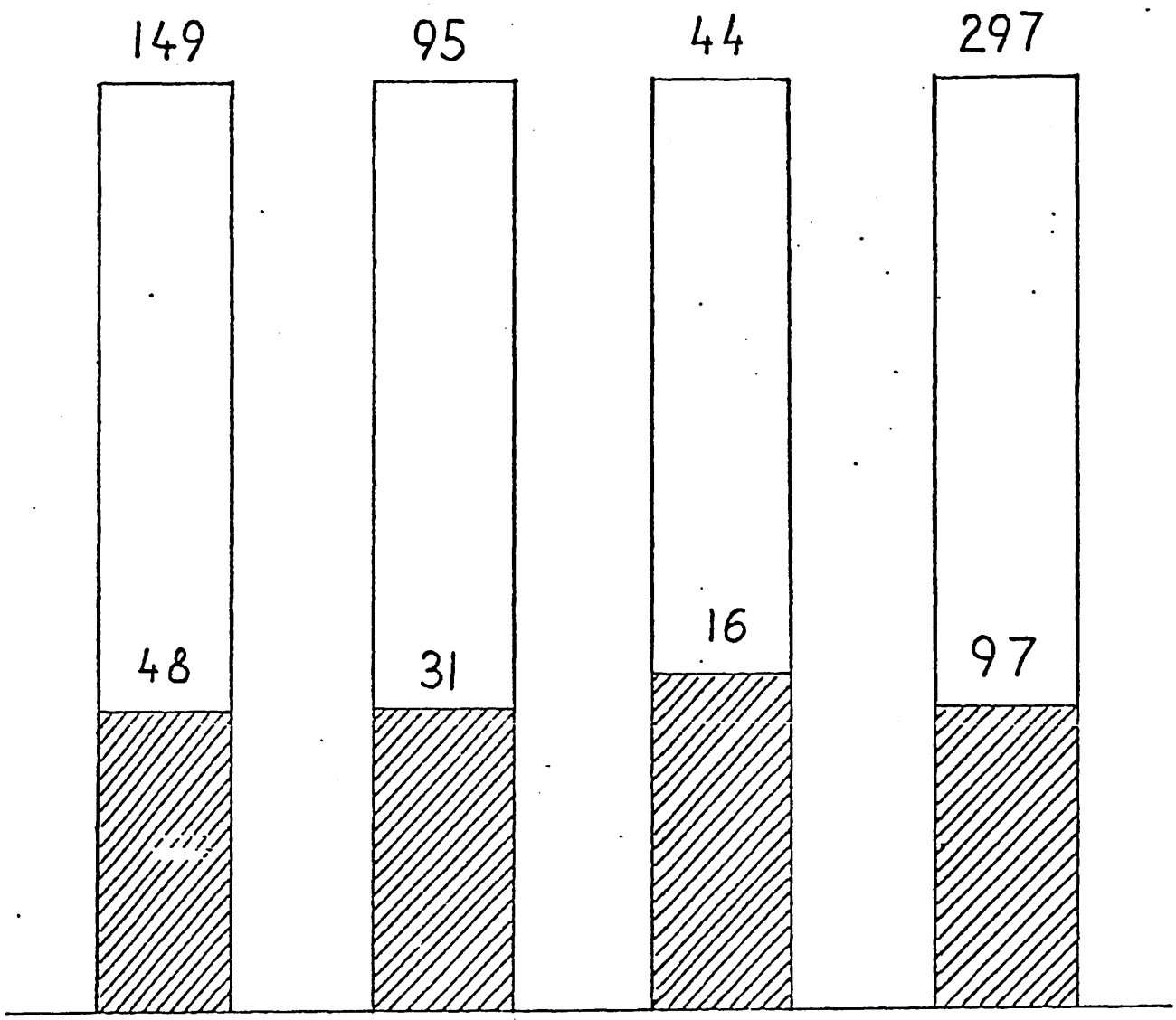



Fig. 5. The frequency of infected plantations of different pines in New Brunswick - 1978.

(Aug. 31)

Table 3

Occurrence of Scleroderris canker by host

in the five New Brunswick forest regions

| Region | Host infected | | | Other | Total |
|---|---|---|---|---|---|
| | jP | rP | scP | | |
| 1. Northeast | 14 | 8 | - | - | 22 |
| 2. Central | 11 | 12 | 10 | 1 | 34 |
| 3. Southeast | 8 | 1 | 2 | - | 11 |
| 4. Southwest | 6 | 7 | 3 | 1 | 17 |
| 5. Northwest | 9 | 3 | 1 | - | 13 |
| Total | 48 | 31 | 16 | 2 | 97 |

Table 4

"Severity rating" of infected plantations by host in New Brunswick - 1978

| Host | High | Moderate | Low | Trace | Unrated |
|---|---|---|---|---|---|
| Jack pine | 17 | 7 | 9 | 2 | 13 |
| Red pine | 4 | 10 | 12 | 1 | 4 |
| Scots pine | 5 | 6 | 1 | - | 4 |
| All hosts | 26 | 23 | 22 | 3 | 23[a] |

a/ includes the 2 unrated "other" plantations

Plantations in different severity rating classes based on rated plantations by host

| | Percentage in different classes | | | |
|---|---|---|---|---|
| Host | High | Moderate | Low | Trace |
| Jack Pine | 48.6 | 20.0 | 25.7 | 5.7 |
| Red Pine | 14.8 | 37.0 | 44.5 | 3.7 |
| Scots Pine | 41.7 | 50.0 | 8.3 | - |
| All hosts | 35.1 | 31.1 | 29.7 | 4.1 |

Severity rating (adopted from Gross and Buchan).

| Level | Percentage of trees affected |
|---|---|
| High | More than 25% |
| Moderate | More than 5% to High |
| Low | More than 1% to Moderate |
| Trace | More than 0% to Low |

a? after the rating indicates that the level of infection in at least some "pockets" in the plantation is higher than shown.

Table 5

Locations where Scleroderris canker was found in 1978

NBDNR Region 1 Northeast

| County | Location | UTM Grid | FIDS No 78-2 | Host | Owner | Rating |
|---|---|---|---|---|---|---|
| Rest. | Blackland | 19-70-531 | 0745 | rP | Z.Comeau | L |
| Rest. | Lorne | 19-71-530 | 0691 | jP | DNR | L? |
| Rest. | St.Jean Baptiste-de-Rest. | 19-63-529 | 0440 | jP | ? | ? |
| Rest. | St.Jean Baptiste-de-Rest. | 19-63-528 | 0200 | jP | DNR? | L? |
| Rest. | Whites Brook | 19-63-528 | 0477 | jP | DNR | H? |
| Rest. | Mann, Robinson Brook | 19-63-528 | 0398 | jP | ? | H |
| Rest. | Guercheville,Five Fingers Bk. | 19-62-527 | 0399 | rP | ? | L |
| Glou. | Butte-d'Or | 20-33-526 | 0435 | rP | DNR | M? |
| Glou | Butte-d'Or | 20-33-526 | 0417 | rP | DNR | ? |
| North. | Indian Falls | 19-70-525 | 0513 | jP | DNR | Nat. Forest |
| Glou | Brunswick Mines, Nine Mi. Rd. | 20-27-525 | 0681 | jP | DNR | L? |
| Glou. | Allardville, Victors Rd. | 20-31-525 | 0549 | rP | DNR | L |
| Glou. | Allardville | 20-31-525 | 0594 | rP | DNR | ? |
| Glou. | Allardville | 20-32-525 | 0539 | rP | DNR | T? |
| Glou. | Allardville, Acadia Rd. | 20-32-525 | 0602 | jP | DNR | H |
| Glou. | Tracadie Artillery Range | 20-34-525 | 0495 | jP | DNR | L |
| North | Little Bald Mountain | 19-69-524 | 0509 | jP | DNR | Natural Forest |
| North. | Little Bald Mountain | 19-69-524 | 0512 | jP | CFS | H |
| North. | Little Bald Mountain | 19-69-524 | 0509 | jP | CFS | H |
| Glou | Jeanne-Mance | 20-31-524 | 0599 | jP | DNR | L? |
| Glou | Jeanne-Mance | 20-31-524 | 0163 | jP | DNR | H |
| Glou. | Jeanne-Mance | 20-31-524 | 0689 | rP | DNR | H |

Table 6

Locations where Scleroderris canker was found in 1978

NBDNR Region 2 - Central

| County | Location | UTM Grid | FIDS No 78-2 | Host | Owner | Rating |
|---|---|---|---|---|---|---|
| North. | Heath Steele | 19-72-524 | 0505 | jP | DNR | Nat. forest |
| North | Heath Steele Mines | 19-72-524 | 0548 | rP | DNR | M |
| North | Bushby | 20-30-522 | 0619 | rP | DNR | L? |
| North | Eel River Bridge | 20-34-521 | 0198 | rP(jP) | DNR | M |
| North | Whitney | 20-29-520 | 0159 | scP | F.Menzies | L? |
| North | Douglasfield | 20-310520 | 0808 | scP | W.Trevors | M |
| North | Redmondville | 20-320520 | 0511 | jP | ? | H |
| North | Hexam | 20-33-520 | 0419 | scP | DNR | Hedge |
| North | Weldfield road | 20-31-519 | 0461 | rP | DNR | L? |
| North | Blackville Lr. Dungarvon burn | 20-27-518 | 0436 | jP | Ont-Minn.Ltd. | M |
| Kent | Koughibouguac Nat. Park | 20-35-518 | 1168 | P | Parks Canada | ? |
| North | Big Hole Brook Rd. | 19-69-517 | 0312 | rP | DNR | ? |
| North | Cains River, mouth of | 20-28-517 | 0278 | jP | DNR | Nat. forest |
| North | Blackville Fire Tower | 20-29-517 | 0479 | jP | DNR | H |
| North | Blackville Fire Tower | 20-29-517 | 0480 | jP | DNR | M |
| North | Blackville Fire Tower | 20-29-517 | 1064 | scP | Acadia? | ? |
| Kent | Acadieville | 20-32-517 | 0441 | jP | Irving | M? |
| Kent | Kouchibouguac Nat. Park | 20-35-517 | 0510 | jP | Parks Canada | H |
| North | Blackville | 20-28-516 | 0161 | scP | W.Morehouse | M |
| North | Upper Blackville | 20-28-516 | 0160 | rP | W. Morehouse | L? |
| North | Blackville Fire Tower | 20-29-516 | 0493 | rP | DNR | L |
| North | Rogersville | 20-30-516 | 0306 | rP | DNR | L? |
| North | Rogersville | 20-30-516 | 0305 | rP | DNR | L |
| North | Rogersville | 20-30-516 | 0307 | rP | DNR | M |
| North | Despres Lake | 20-30-516 | 0265 | jP | DNR | M? |
| North | Despres Lake | 20-30-516 | 0660 | rP | DNR | L |
| North | Despres Lake | 20-30-516 | 0661 | jP | DNR | M |
| York | Hayesville | 19-68-515 | 0196 | rP | S. Hunter | M |
| North | Porter Brook | 19-68-515 | 0195 | scP | G. Stanton | H |
| Kent | St-Pierre | 20-36-515 | 0533 | scP | Hofert | H |
| York | Parker Ridge | 19-68-514 | 0627 | scP | ? | ? |
| York | Astle Station | 19-69-514 | 0574 | scP | H. Munn | M |
| North | Bettsburg | 19-71-514 | 0259 | scP | A. Parker | M |
| North | Shinnickburn | 20-27-514 | 0239 | jP | Acadia? | ? |

Table 7

Locations where Scleroderris canker was found in 1978

NBDNR Region 3 - Southeast

| County | Location | UTM Grid | FIDS No. 78-2- | Host | Owner | Rating |
|---|---|---|---|---|---|---|
| Kent | Upper Main River | 20-34-515 | 0534 | scP | Hofert | M |
| Kent | ? | 20-36-515 | 0533 | scP | Hofert | M |
| Kent | St.-Edouard-de Kent | 20-36-515 | 0401 | jP | Irving | T? |
| Kent | St. Pierre | 20-36-515 | 0340 | jP | Irving | H |
| Kent | St. Maurice | 20-36-515 | 0345 | jP | Irving | L? |
| Kent | St. Norbert | 20-35-514 | 0536 | jP | Irving | ? |
| Kent | St. Norbert | 20-35-514 | 0532 | jP | Irving | H |
| Kent | St. Maurice | 20-36-514 | 0535 | rP | Irving | M |
| Kent | St. Maurice | 20-36-514 | 0537 | jP | Irving | H |
| Queens | Upper Gaspereau | 20-28-513 | 0452 | jP | ? | random |
| Kings | Walton Lake | 20-31-505 | 0124 | jP | ? | ? |

Table 8

Locations where Scleroderris canker was found in 1978

NBDNR Region 4 - Southwest

| County | Location | UTM Grid | FIDS No. 78-2 | Host | Owner | Rating | |
|---|---|---|---|---|---|---|---|
| Carleton | Juniper, McKiel Brook | 19-65-516 | 0473 | jP | Irving | H | |
| Carleton | Juniper,McKiel Brook | 19-65-515 | 0092 | jP | Irving | ? | |
| Carleton | Juniper, Nursery | 19-63-515 | 0234 | rP | Irving | H | windbreak |
| Carleton | Juniper, Nursery | 19-63-515 | 0116 | rP | Irving | H | 2-0 |
| Carleton | Juniper, Nursery | 19-63-515 | 0121 | rP | Irving | H | 3-0 |
| Carleton | Juniper, Nursery | 19-63-515 | 0235 | rP | Irving | M | plantation |
| Carleton | Juniper, Airstrip | 19-64-515 | 0232 | jP | Irving | ? | |
| Carleton | Juniper, S. of | 19-63-515 | 0472 | rP | Irving | L? | |
| Carleton | The Forks | 19-63-515 | 0233 | jP | Irving | T? | |
| York | Deersdale | 19-64-515 | 0491 | jP | Irving | H | |
| York | South Portage, Glencoe Rd. | 19-68-512 | 0191 | scP | Young | H | |
| York | Nashwaak Bridge, Forbes Field | 19-68-512 | 0610 | scP | McKay | H | |
| York | Nashwaak Bridge, Woodcock | 19-68-512 | 0609 | scP | McKay | H | |
| Sun. | Acadia For. Exp. Sta. | 19-69-509 | 0342 | jP | CFS | M? | |
| York | Cork Station, Hanwell Rd. | 19-69-506 | 0257 | P | Bidlake | ? | |
| York | Upper Brockway | 19-64-504 | 0657 | rP | Georgia Pacific | M | |
| Charlotte | Oak Bay | 19-64-501 | 0260 | rP | ? | M? | |

Table 9

Locations where Scleroderris canker was found in 1978

NBDNR Region 5 - Northwest

| County | Location | UTM Grid | FIDS No. 78-2 | Host | Owner | Rating |
|---|---|---|---|---|---|---|
| Rest. | Summit Depot | 19-55-529 | 0418 | jP | F.C.L. | H |
| Mad. | North Rd. to Guonamitz Rd. | 19-59-526 | 0176 | jP | Irving | ? |
| Mad. | St. Leonard - Skin Gulch Rd. | 19-59-525 | 0171 | jP | Irving | H |
| Mad. | St. Leonard - Skin Gulch Rd. | 19-59-525 | 0172 | jP | Irving | ? |
| Mad. | St. Leonard - Landing | 19-59-525 | 0173 | jP | Irving | M? |
| Mad. | St. Leonard - Ouellette Rd. | 19-59-525 | 0169 | jP | Irving | L |
| Vict. | Boston Brook Gate, E. of | 19-60-525 | 0186 | jP | Irving | L? |
| Vict. | St. Leonard-Old Rest. Rd. | 19-60-525 | 0170 | jP | Irving | L? |
| Vict. | Black Brook Gate, E. of | 19-61-524 | 0187 | rP | Irving | L? |
| Vict. | Black Brook Gate, E. of | 19-61-524 | 0189 | jP | Irving | H |
| Mad. | Saint-Francois-de-Madawaska | 19-52-523 | 0475 | rP | Nadeau | ? |
| Vict. | Boston Brook Gate, S. of | 19-60-523 | 0185 | rP | Irving | M. |
| Vict. | Grand Falls, portage | 19-59-520 | 0688 | scP | Private | ? |