Distribution of Serological Strains of *Gremmeniella abietina* in Eastern North America

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ABSTRACT

Skilling, D., Kienzler, M., and Haynes E. 1984. Distribution of serological strains of *Gremmeniella abietina* in eastern North America. Plant Disease 68:937-938.

A total of 327 mycelial isolates of *Gremmeniella abietina* from northern North America were serologically typed to determine fungus strain. All but six were either the North American or European strain. Isolates from Michigan, Minnesota, and Wisconsin were all typed as North American strain. The European strain predominated in New York, Vermont, and Newfoundland isolates.

In 1977, Dorworth (4) reported that the population of *Gremmeniella abietina* (Lagerb.) Morelet in New York State was composed of two strains that could be separated by their immunogenic reactions. One strain, called the North American (NA), has been present in North America since at least the early 1950s (15). This strain is characterized by usually infecting branches lower than 2 m above the ground, by a host range primarily limited to *Pinus*, and by its production of abundant ascospores and conidia. The second strain is characterized by occurrence on branches at any height; by a host range that includes *Pinus, Picea, Abies, Larix, Tsuga,* and *Pseudotsuga*; and by very limited ascospore production under New York conditions. It is serologically identical to isolates of *G. abietina* from Europe and thus was called the European (EU) strain (4).

Because the EU strain of *G. abietina* is believed to have been introduced to the United States, its potential for causing damage to forests of North America is unclear. Reports of its greater virulence as indicated by ability to attack trees of all sizes and in several genera created concern among forest managers. In response to this concern, several plant quarantines were established in 1977. These included state quarantines in New York and Vermont, a federal regulatory action, and a general quarantine by the Canadian government on plant material from all countries where the EU strain is present. The major regulatory problem was perceived to be shipment of Christmas trees, which might harbor the pathogen (8), throughout eastern North America. Because most of the quarantines were against the EU strain, it was necessary to identify the strain of *G. abietina* in new infection centers. A serology laboratory was developed by the North Central Forest Experiment Station at St. Paul, MN, to make these determinations. This paper gives the immunogenic reactions of the isolates tested in eastern North America in relation to locality of origin.

Accepted for publication 13 June 1984 (submitted for electronic processing).

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MATERIALS AND METHODS

Samples were collected during the spring and summer from 1977 to 1983. Most samples were taken from branch tips showing dieback typical of that caused by *G. abietina*. Most of the samples were collected from red pine (*Pinus resinosa* Ait.) and Scots pine (*P. sylvestris* L.). A few samples were collected from jack pine (*P. banksiana* Lamb.) and one sample each was collected from black spruce (*Picea mariana* (Mill.) B.S.P.) and ponderosa pine (*Pinus ponderosa* Laws.). The samples were refrigerated whenever possible between collection and isolation. When received at the North Central Forest Experiment Station, the samples were maintained at 5 C until isolation. *G. abietina* was isolated from infected branch samples by surface-sterilizing 5-mm^2 wood chips in a 0.5% solution of sodium hypochlorite for 1 min or by aseptically removing conidia from pycnidia on branches. The chips or conidia were placed on a malt agar medium containing 150 ml of V-8 juice per liter and incubated at 5 C in darkness.

Subcultures taken from the original isolations were incubated at 17 C and retransferred until pure cultures of *G. abietina* were obtained. When the colonies were about 50 mm in diameter, 5-mm^2 mycelial plugs were transferred to flasks containing filtered V-8 juice (200 ml/L) and glucose (20 g/L). The flasks were incubated in darkness at 17 C and shaken daily to aerate the mycelium and break up the mycelial mat. After 21 days, the mycelium was homogenized in phosphate buffer in a cell homogenizer. Soluble proteins were filtered from the homogenized mycelium using a Millipore filter assembly and used as antigens in tests for strain determination by gel double diffusion (1). Two NA antisera were prepared using isolates SC-1 and SC-5 of *G. abietina* collected from *P. resinosa* in Ontario. Two EU antisera were prepared using isolates SF-4, collected from *Larix sibirica* Ladeb. in Finland, and 17-34, collected from *P. sylvestris* in New York State. Each antigen was allowed to react with NA antiserum SC-1 and EU antiserum SF-4. In cases of anomolous reaction, the antigens were tested further with NA antiserum SC-5 and EU antiserum 17-34. Procedures were described in detail by Skilling and Kienzler (14).

RESULTS AND DISCUSSION

We classified 327 isolates of *G. abietina* by their immunogenic reaction (Table 1). All 33 isolates from Michigan, Minnesota, and Wisconsin reacted positively to NA antiserum. Of the 112 isolates from New York State, 96 tested as EU and 15 as NA. One isolate from New York reacted positively to both NA and EU antisera (12).

The EU strain predominated in Vermont, with 63 isolates giving EU reactions. Both strains were present in Maine, although the sample size was too small to make any conclusions about distribution. The EU strain also predominated in Newfoundland. Samples from Quebec were about equally divided between NA and EU. One isolate from black spruce from Laurentide Park in Quebec tested as EU strain. The Laurentide Park branch sample used for isolation bore numerous apothecia of *G.*

Table 1. Immunogenic reactions of isolates of *Gremmeniella abietina* in relation to locality of origin

Location	Isolates tested	Strain type[a]		
		European	North American	Intermediate
New York	112	96	15	1
Vermont	70	63	7	0
New Hampshire	2	2	0	0
Maine	2	1	1	0
Quebec	46	23	22	1
New Brunswick	51	18	31	2
Newfoundland	10	9	0	1
Nova Scotia	1	0	0	1
Michigan	14	0	14	0
Minnesota	3	0	3	0
Wisconsin	16	0	16	0
Totals	327	212	109	6

[a] All isolates were tested with antisera prepared from both North American and European isolates of *G. abietina*. Isolates giving a positive response to both NA and EU antisera were typed as intermediate.

abietina. These are rarely found on conifer species infected with the EU strain. One sample from Quebec gave a positive immunogenic reaction to both NA and EU antisera. New Brunswick also had both strains present, with the NA strain predominating.

This study showed that the EU strain predominates in New York and Vermont. The EU strain was also identified along the southern border of Quebec, but only the NA strain was detected in samples from the remainder of the province. Most samples from Newfoundland also typed as EU strain but the sample size was not large enough to make conclusions. None of the Lake States samples typed as EU strain. In all areas sampled, the distribution pattern based on immunogenic reaction agrees with the distribution pattern based on infection height and number of host genera infected.

The NA strain, which is present across the northern United States and southern Canada (2,13), was first identified in about 1950 in the Upper Peninsula of Michigan and near Sault Ste. Marie, Ontario. Since that time, the fungus has spread by windborne ascospores (2) and on infected nursery stock (9). Isolates from both Europe and Asia have never given a positive immunogenic reaction to the NA antiserum (3), so we still do not know where the NA strain originated. The EU strain appears to have been present in New York for several years before 1975, when it was identified as differing from the fungus present in the Lake States (10).

One isolate found in New York showed characteristics of both the EU and NA strains. This isolate was found in the upper crowns of large trees, which is characteristic of the EU strain. It produced large quantities of ascospores under field conditions, which is rare for other EU isolates in New York but normal for NA isolates. Single-ascospore cultures derived from one apothecium produced mycelial cultures that gave either NA or EU immunogenic reactions, depending on which single-spore culture was used. This suggests the possibility of hybridization between EU and NA strains (5).

Isolates from Vermont gave either the NA or the EU immunogenic reaction. Wendler et al (17), however, found several Vermont isolates with an immunogenic reaction that they termed intermediate. Their serological procedure was slightly different from ours in that a third antiserum was produced by Wendler in response to a Vermont isolate. This isolate was classified as an intermediate (I) strain. In our tests, an isolate was classified as I strain when it reacted positively to both NA and EU antiserum. One isolate each from New York, Quebec, and Nova Scotia and two isolates from New Brunswick gave the I response.

The isolate from Laurentide Park raises the question of where the EU strain first appeared in North America. This isolate tested as EU but produced abundant apothecia; therefore, it does not completely fit the characteristic pattern of the EU strain. The spruce stand where this isolate originated, as described by Smerlis (16), had been infected for some time before 1964. Thus the Laurentide Park infection may predate infections by the EU strain in New York.

It appears from this and previous studies (6,7,11) that the NA strain of *G. abietina* occurs from Newfoundland to British Columbia. The EU strain is present in northern New York, adjacent Quebec, and the Canadian Maritime Provinces. No information is available as to which strain will predominate when both are present in the same area.

More information is needed on the distribution and significance of *G. abietina* strains, especially on the biological significance of isolates that are immunogenically intermediate between NA and EU. To prevent the possible spread of the EU strain, isolates testing as intermediate should be treated as EU strain until their field pathogenicity can be determined.

ACKNOWLEDGMENTS

We thank the following who supplied samples as a part of disease surveys and regulatory activities: B. Schneider, J. Sullivan, and Don Fasking, New York Department of Environmental Conservation; G. Cooke, New York Department of Agriculture and Markets; J. O'Brien, M. Weeks, and M. K. Robbins, USDA Forest Service; D. Bergdahl, University of Vermont; R. Kelley, Vermont Department of Forests, Parks, and Recreation; A. Prey, Wisconsin Department of Natural Resources; and L. Magasi, D. LaChance, C. Dorworth, and P. Singh, Canadian Forestry Service. Denise DuPont, USDA Forest Service, did many of the early serology tests.

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