Influence of Inoculum Concentration on Infection of Red Pine Seedlings by *Gremmeniella abietina*

C. E. Dorworth

Research scientist, Department of the Environment, Canadian Forestry Service, Great Lakes Forest Research Centre, Sault Ste. Marie, Ontario, Canada P6A 5M7.

I wish to thank B. Payandeh for statistical aid, D. Tyrell for constructive criticism, and C. Davis and Daphne Tracy for technical assistance.

Accepted for publication 22 September 1978.

ABSTRACT

DORWORTH, C. E. 1979. Influence of inoculum concentration on infection of red pine seedlings by Gremmeniella abietina. Phytopathology 69: 298-300.

Inoculation of *Pinus resinosa* seedlings with a graded series of inocula of the North American race of *Gremmeniella abietina* revealed that 2.5×10^8 conidia per square meter of transplant-bed area were required to kill 50% of the seedlings. In general, the capacity of the pathogen to colonize an artificial medium was reduced after a single passage through the host, although 2% of the isolates recovered from the host showed enhanced growth.

Additional key words: breeding for resistance, *Scleroderris lagerbergii*.

Gremmeniella abietina (Lagerb.) Morelet (= *Scleroderris lagerbergii* Gremmen) is a destructive pathogen of *Pinus* spp. and an occasional pathogen of other conifers in parts of North America and Europe (4) and of *Abies sachelinensis* Mast. (21) and *Pinus strobus* L. (19) in Japan. The existence of physiologic races of *G. abietina* has been considered (3,8,9,16,17,19), as has the effect of climate on infection and colonization of the host (3–6,13,14).

The disease caused by this pathogen is so destructive that selection and breeding trials have been initiated in Canada, Germany, Norway, Sweden, and the United States to develop resistant pines for reforestation (1,2,7,12,18,20). Selection of resistant jack pine (*P. banksiana* Lamb.), Scots pine (*P. sylvestris* L.), and Austrian pine (*P. nigra* Arnold) is proceeding from a base of promising field selections. The task of red pine (*P. resinosa* Ait.) selection will undoubtedly be more difficult, as red pine is the most severely damaged North American host and one in which no resistant individuals have been found (7). Red pine, which forms uniform, easily managed plantations, has been used extensively in reforestation efforts as an alternative to conifers more susceptible to damage by fungi and insects (4). The genetic uniformity that has been used to advantage in forest management may prolong the task of selecting and breeding lines resistant to *G. abietina*.

Relation of inoculum density to infection is important because apparent resistance among certain *Pinus* spp. breaks down under extremely high inoculum loads (C. E. Dorworth, *unpublished*). Employment of standard inoculum dosages could permit retention of host seedlings with various degrees of resistance.

The objectives of the present study were to determine: (i) lethal inoculum dosage 50 (LID_{50}), ie, the quantity of spores required to infect half of a population of 3-yr-old red pine seedlings under site conditions encountered commonly in the northern Great Lakes area, and (ii) the extent to which growth rate of the pathogen in vitro was altered by passage through the host.

MATERIALS AND METHODS

Red pine seedlings (2-0 stock) were spring planted near Sault Ste. Marie, Ontario, Canada, in rows of 25, with interseedling spacing of 0.5 m, and were inoculated the following spring with conidia of *G. abietina* isolate C-1 (ATCC 28379, CSB 336.73, DAOM 147882). The design included two blocks, each divided into four replicates, each replicate comprising one row of seedlings per inoculum concentration, for a total of eight rows, or 200 seedlings per inoculum concentration. Concentrations tested were 5×10^6, 2.5×10^6, 1×10^6, 7.5×10^5, 5×10^5, 1×10^5, 5×10^4, 5×10^3, and 0 spores per milliliter. Conidial suspensions were prepared by dispersing spore cirri of isolate C-1 from V8 agar (8) in 0.1% water agar. Spore germination was 98% on water agar. Spore concentration was estimated with a Levy corpuscle counting chamber and dilutions were prepared with 0.1% water agar. Inoculum was sprayed over the seedlings in June 1973 with a Holcumb sprayer (Willex Prods. Ltd., Mississauga, Ontario) from a height of 30 cm above ground (approximately 15 cm from the foliage) at a rate of 1 ml per seedling, within a shield to impede drift. Seedling shoots were partially extended by this time. Seedlings were not covered following inoculation. Intermittent light rain and continual high relative humidity during the following 24 hr provided conditions necessary to insure infection (13) at the temperature range (15–20 C) in the experimental area.

The percentage of infected seedlings was recorded the following spring (seedlings remained asymptomatic during the season of inoculation) until no additional seedlings showed symptoms (ie, epinasty, orange discoloration of needle base). Infected seedlings were surface-disinfested for 1 min with 2.5% NaClO containing a trace of Tween 20, then dissected aseptically. Samples from the interface of necrotic and visibly healthy tissue of each seedling were placed on V8 agar in petri dishes and incubated at 20 C. One culture per infected seedling was retained by monoconidial transfer to a van Tiegham cell in V8 medium.

The growth of 75 isolates after 5 wk of incubation, defined as total colony area $\pm$ 5 mm^2, was recorded (8). Of these isolates, 70 were chosen at random (Group III) and five that did not conform in color, colony form, or colony size to the group of isolates as a whole were selected as possible variants (Group II). Five subcultures of the original isolate C-1 were used for comparison (Group I). Each culture or subculture was replicated on 10 plates. The data were subjected to analysis of variance, and the mean colony area of Group I was compared with that of the isolates as a whole and with the means of Groups II and III by the individual degrees of freedom test (11).

00031/949X/79/000054$03.00/0

RESULTS

Because red pine seedlings seldom recover from infection with *G. abietina*, the percentage of infected seedlings was translated directly as seedling mortality. Fifty percent of the seedlings inoculated with 2.5×10^6 propagules per seedling became infected ($LID_{50} = 2.5 \times 10^6$) (Fig. 1).

A 3-yr-old open-grown red pine seedling occupies approximately 100 cm^2 of bed space. This translates roughly to 2.5×10^8 propagules per square meter of transplant bed required to achieve 50% mortality in a single application under field conditions. Mortality approaches 100% at 5×10^8 propagules per square meter.

The LID_{50} value was calculated from symptoms alone. Only about 70% of the seedlings with symptoms yielded pure cultures of *G. abietina*. Presence of the pathogen in the remaining 30% of apparently diseased seedlings was inferred from superficial colony characteristics in mixed cultures. None of the seedlings to which water agar alone was applied became infected.

Group III isolates colonized significantly less of the surface of the culture medium than did Group I stock isolates (Table 1). Group II isolates, selected on the basis of visually detectable aberrant growth, yielded significantly larger colonies than did the stock cultures.

TABLE 1. Colony size of *Gremmeniella abietina* isolates from several sources in culture

Isolate source	Degrees of freedom	Coefficient of variation (%)	Mean colony area[a] (mm^2)
Group I[b]	50	30.8	1,005
Group II[c]	50	63.7	1,674
Group III[d]	700	31.8	868

[a] Braces join significantly different ($P = 0.01$) means.
[b] Group I = stock cultures of isolate C-1.
[c] Group II = visually selected cultural aberrants.
[d] Group III = isolates selected at random after passage through host.

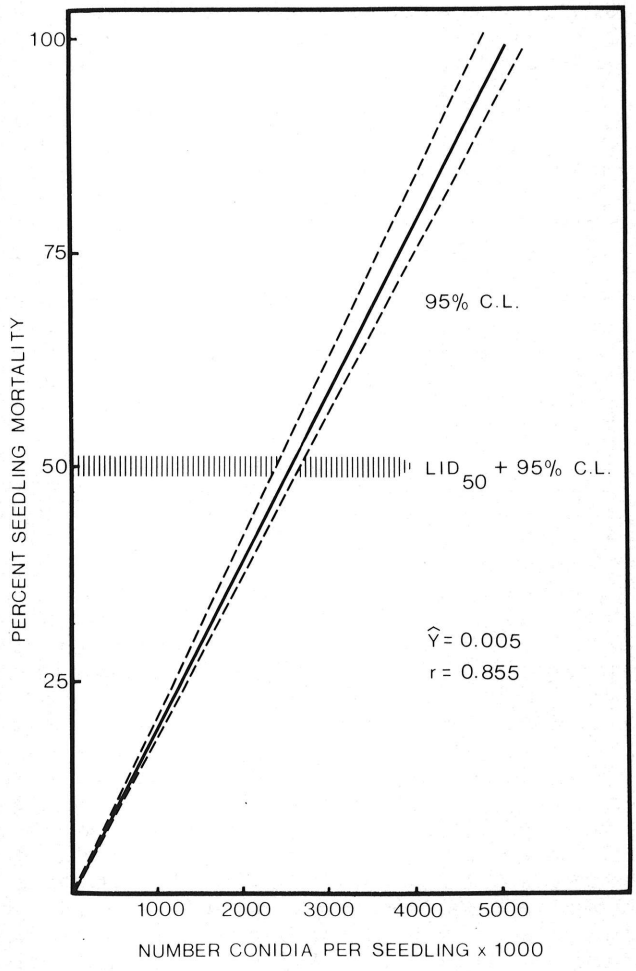

Fig. 1. Regression of mortality of 3-yr-old *Pinus resinosa* following inoculation with different concentrations of inoculum of *Gremmeniella abietina*. C.L. = confidence limits; LID_{50} = lethal inoculum dosage 50.

DISCUSSION

Immediate applications of the outlined technique include standardization of inoculum for experimental work and interpretation of nursery disease situations. The results are germane to infection caused both by ascospores and by conidia, as the two spore states are equally infective (6,10). Each is produced in abundance by the North American race of *G. abietina*, although the sexual state is rarely produced by the European race (4,8). The North American race infects pine meristems from ground level to a height of approximately 2 m and causes formation of occasional basal stem cankers, which are the reason for the local common name, Gremmeniella canker. The European race, by contrast, has been known for nearly 100 years in Europe as a dieback disease of both immature and mature pines ("Kieferntriebsterben") and of other conifers (3,4,12). A similar or identical race (9) recently was implicated as the cause of a mature tree dieback in the United States that resembles closely the European syndrome (15). Results of the present study should not be applied to field situations where the dieback syndrome exists unless host response to the two races is shown to be identical.

Growth measurements of many *G. abietina* isolates maintained in culture for a number of years yielded reproducible growth curves useful in intraspecies comparison (8). A culture of C-1 that had been maintained on agar for 2 yr in the earlier test had twice the growth potential as the same culture newly recovered from pine in the present test (Table 1). Newly recovered isolates and those maintained in prolonged laboratory culture represent separate populations in terms of growth rate, and this must be considered in any attempt to use growth rate as a means of isolate characterization.

As field testing is invariably complicated by uncontrolled variables, employment of a single source of inoculum and specified inoculum loads will provide a basis for comparison among tests conducted in different areas. This will also provide a uniform basis for second-phase screening with other isolates to broaden the scope of resistance and for possible later definition of pathogenic races of *G. abietina*.

LITERATURE CITED

1. BJÖRKMAN, E. 1972. Die Prüfung forstlicher Baumarten auf Resistenz gegen parasitäre Pilze. Eur. J. For. Pathol. 2:229-237.
2. DIETRICHSON, J. 1968. Provenance and resistance to Scleroderris lagerbergii Gremmen (Crumenula abietina Lagerb.). The international Scots pine provenance experiment of 1938 at Matrand. Medd. Nor. Skogforsøksves. 25:395-410.
3. DONAUBAUER, E. 1974. Über das Kieferntriebsterben in Österreich. Pages 67-98 *in* 100 Jahre Forstliche Bundesversuchsanstalt. Forstl. Bundesversuchsanstalt, Vienna.
4. DORWORTH, C. E. 1971. Diseases of conifers incited by Scleroderris lagerbergii Gremmen: A review and analysis. Can. For. Serv. Publ. 1289. 42 pp.
5. DORWORTH, C. E. 1972. Epidemiology of Scleroderris lagerbergii in central Ontario. Can. J. Bot. 50:751-765.
6. DORWORTH, C. E. 1973. Epiphytology of Scleroderris lagerbergii in a kettle frost pocket. Eur. J. For. Pathol. 3:232-242.
7. DORWORTH, C. E. 1974. Disease problems in intensively managed forests—Scleroderris lagerbergii—Possibilities for selection and breeding for resistance. Eur. J. For Pathol. 4:228-232.
8. 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. Bot. 53:2506-2525.

9. 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. Rep. 61:887-890.
10. GREMMEN, J. 1968. Bijdrage tot de biologie van Brunchorstia pinea (Karst.) Höhn., de oorzaak van het taksterven bij Oostenrijkse en Corsicaanse den. Ned. Bosbouw. Tijdschr. 40:221-231.
11. LI, J. C. R. 1957. Introduction to Statistical Inference. Edwards Bros., Inc., Ann Arbor, MI. 568 pp.
12. ROLL-HANSEN, F. 1972. Scleroderris lagerbergii: Resistance and differences in attack between pine species and provenances—A literature review. Eur. J. For. Pathol. 2:26-39.
13. SKILLING, D. D. 1969. The effect of temperature on ascospore release by Scleroderris lagerbergii. Plant Dis. Rep. 53:289-291.
14. SKILLING, D. D. 1969. Spore dispersal by Scleroderris lagerbergii under nursery and plantation conditions. Plant Dis. Rep. 53:291-295.
15. SKILLING, D. D. 1977. The development of a more virulent strain of Scleroderris lagerbergii in New York State. Eur. J. For. Pathol. 7:297-302.
16. SMERLIS, E. 1976. Evaluation of fungicides for control of Gremmeniella abietina. I. Laboratory and preliminary field assays. Can. For. Serv. Inf. Rep. LAU-X-23. 9 pp.
17. STEPHAN, B. R. 1970. Beitrag zu der durch Scleroderris lagerbergii verursachten Triebsptizenkrankheit an verschiedenen Kiefernarten. Pflanzenkr. Pflanzenschutz 77:417-424.
18. STEPHAN, B. R. 1970. Klonabhängiges Verhalten bei Pinus nigra Arnold gegenüber Scleroderris lagerbergii Gremmen. Allg. Forst. Jagdztg. 141:60-63.
19. TAKAHASHI, I. 1973. Scleroderris lagerbergii Gremmen found on planted Pinus strobus L. Proc. Annu. Meet. Jpn. For. Soc., Hokkaido Br. 22:106-110.
20. TEICH, A. H., and E. SMERLIS. 1969. Jack pine resistance to Scleroderris lagerbergii. Can. For. Serv. Bi-mon. Res. Notes 25:47.
21. 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. Eur. J. For. Pathol. 4:65-74.