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Conditions conducive to an epidemic of *Gremmeniella abietina*, European race, in red pine plantations

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ABSTRACT: In North America, *Gremmeniella abietina*, European race (GaEU), was reported in 1975. Our objective was to follow the spread of GaEU on red pines growing on flat land and on slope. Annual height infection varied significantly on flat land, ranging from 60 to 110 cm in 1991 to 0 to 50 cm in 1992. On the slope, pines in the bottom were killed by the disease, but survived on the top. Favorable conditions follow a horizontal line about 10 m over the lower elevation and are probably related to fog or mist. The horizontal disease spread over a 3-year period was only 20 m and this is mainly explained by the absence of ascospores in North America.

Keywords: climatic conditions; epidemic; *Pinus resinosa*; Scleroderris canker

The fungal pathogen *Gremmeniella abietina* (Lagerb.) Morelet causes shoot blights and cankers mainly on two-needle pines. In North America, the European race of *G. abietina* was first reported in New York State and later in Quebec, Canada. The name "European race" was given to distinguish this exotic pathogen from the native North American one. In 1983 and 1984, a survey was conducted in 1183 pine plantations in the north of the Ottawa River, up to the town of Mont-Laurier, and GaEU was found in 121 plantations. Some of the latter were selected to conduct assays for controlling the disease and for identifying factors conducive to an epidemic. The present paper reports observations and measurements in these plantations where our main objectives were: (*i*) to follow disease development in the crown of red pine located on a flat land; (*ii*) to measure the height of disease damage in red pine growing on a land with a slope; (*iii*) to measure the distance of the horizontal spread of the disease from infected trees to healthy red pines.

MATERIAL AND METHODS

Observations and measurements were conducted on three different sites.

Site 1. To follow the spread of the disease caused by GaEU on red pines growing on a flat land, we selected a plantation located at La Macaza (46°20'N; 74°46'W). Approximately 72,000 red pines were planted between 1965 and 1975, from west to east (Fig. 1). The disease was detected in 1983 on the east site, and it had been present there for more than 5 years. It began on the youngest pines on the east side of the plantation where tree mortality was already occurring. It was introduced through infected seedlings planted after 1970 based on previous information (Laflamme 1999). To evaluate the health status of pines, four permanent survey lines of 50 trees were located in 1984 at a distance of 85 m from each other. Line 1 was in the most affected section of the plantation while the other three were on the other side of a road separating the plantation into two parts (Fig. 1). From 1984

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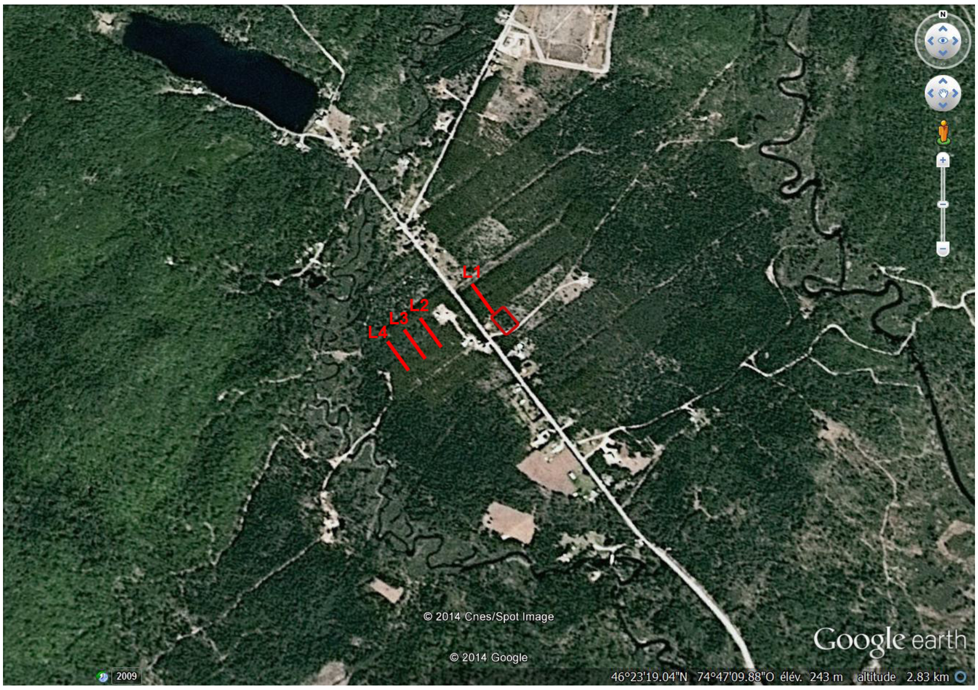

Fig. 1. Localisation of the red pine plantation at La Macaza. The four survey lines L1, L2, L3 and L4 are in Site 1. Site 3 is represented by a rectangle in the field adjacent to site 1

to 1989, visual evaluations of these trees were carried out annually and the health status was noted for each third of the height. From 1990 to 1997, more information was collected: the height of the infection on each pine was measured annually with a pole to the nearest ± 0.1 m; total height of the trees and height of the lower green crown were measured in 1990 and in 1995. A ladder was used to detect pycnidia on the shoots in the crown of trees.

Site 2. To follow the spread of the disease on pines growing on a slope, we selected a plantation located on a hill side at Sainte-Anne-du-Lac (46°52'N; 75°17'W). We estimated the size of the plantation at about 35,000 red pines planted on a slope

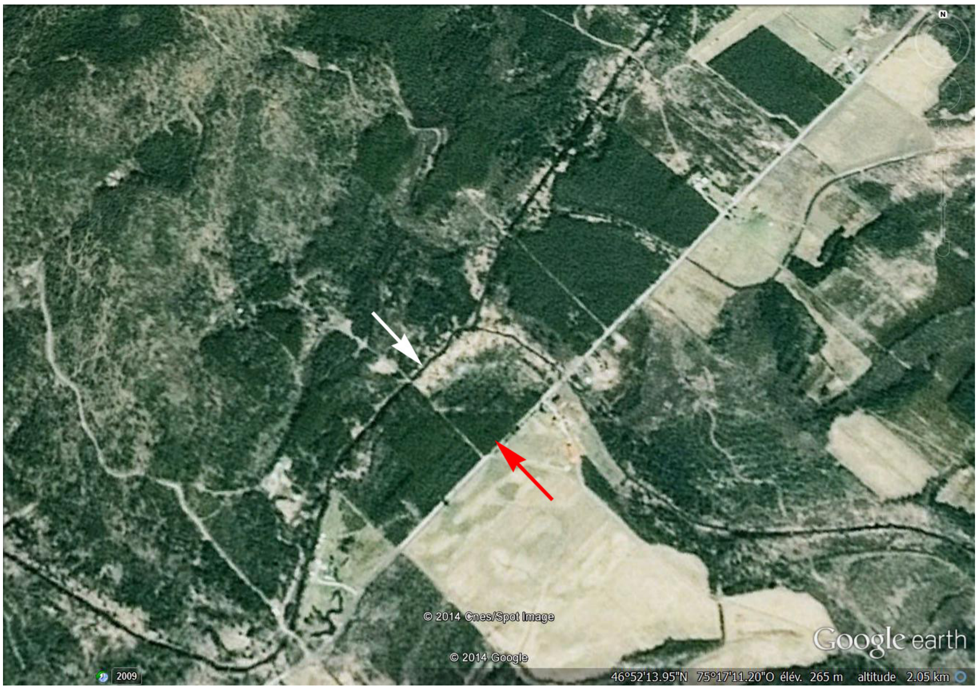

Fig. 2. Localisation of the red pine plantation, Site 2, at Sainte-Anne-du-Lac. The study area is limited by the road (red arrow) and the river (white arrow). Pines surviving to the disease form a triangle on the top of the hill (red arrow)

Table 1. Percentage of trees per survey line infected by *G. abietina* in the lower crown only (LC) or up to the higher crown (HC) at La Macaza (site 1). Lower crown represents 2/3 of the crown and HC the last 1/3

	Survey Line							
	1 (31)*		2 (33)		3 (33)		4 (30)	
	LC	HC	LC	HC	LC	HC	LC	HC
1984	48	52	100	0	100	0	100	0
1985	55	45	61	30	85	15	93	7
1986	55	45	45	45	82	18	90	7
1987	31	58	45	42	88	12	97	3

*number of trees in parentheses

going down from a road towards a river (Fig. 2). The disease was first detected in 1983. At that time, the pines were 14 years old and *G. abietina* was already present on lower branches up to 1.5 m in the whole plantation. To evaluate the health status of pines, two permanent survey lines of 50 trees were established in 1984, one line in the upper part and the other in the lower section. From 1984 to 1989, visual evaluations of the living crown were carried out like on site 1. After 1991, as *G. abietina* had killed all red pines in the bottom part of the hill, measurements were abandoned. To better understand epidemic development, the topography was surveyed in 2003 between the road and the river (Fig. 2). The height between the ground and the base of the living crown was measured on residual pines.

Site 3. To measure spore dispersal, red pine seedlings were planted in a field next to the La Macaza plantation (site 1) in the autumn of 1997 (Fig. 1). Four-year-old seedlings were planted in five rows perpendicularly to the plantation at the level of survey line 1. The distance between rows was 5 m and 15 seedlings were planted 5 m apart in each row for a total distance of 75 m away from pines infected with *G. abietina*. These seedlings were examined for shoot blights annually in July during the next three years. Shoots showing symptoms were collected for laboratory examination, and the race of the pathogen was identified using the method described in Hamelin et al. (2000).

Statistical analysis. Statistical analyses were done using the program R version 3.0.0 (The R Foundation for Statistical Computing 2013). Analyses of variance (ANOVA) were carried out according to the general linear model's procedure and Tukey's tests.

RESULTS

Site 1

A thinning done in 1985 had removed several trees in each survey line, so their number went from 50 down to 30–33 trees per plot. Visual assessment of symptoms was only carried out from 1984 to 1987. Reliable evaluation of tree health from the ground was impossible in 1988 and 1989 because of tree size and the presence of *Pityophthorus puberulus,* an insect causing symptoms similar to scleroderris canker.

In 1984, the disease was present on lower crowns in all survey lines, but the pathogen was detected high into the crowns only in survey line 1 (Table 1). The disease generally progressed along the height from 1985 to 1987. In line 1, the percentage of infected higher crowns continued to increase, while that of the other lines declined from 1986 to 1987 (Table 1).

In 1995, the disease affected all pines with the exception of survey line 4, where symptoms were seen on only 35% of the trees (Table 2). That same year, mortality rates were 19 and 21% in lines 1 and 2, respectively, while it was only 4% in lines 3 and 4. In line 1, 71% of the trees were severely cankered while in the other lines, these rates were much lower and decreasing from line 2 to line 4 (Table 2).

Table 2. Health status (%) of red pines per survey line at La Macaza (site 1)

Pine status	Diseased				Dead				Cankered			
Survey line	1	2	3	4	1	2	3	4	1	2	3	4
1990	97	75	60	35	16	12	0	0	71	0	0	0
1995	100	100	100	35	19	21	4	4	71	12	8	4

Diseased trees showed shoot blight with pycnidia, dead trees had no green foliage, and cankered trees had damaged trunks. The number of living trees in 1990 was 31, 24, 23 and 23 for line 1, 2, 3 and 4, respectively

Table 3. Mean height (m) of the lower part of the trunk without green foliage, of the highest infected shoot found in the crown, and of trees at La Macaza (site 1)

Survey line	Without green foliage				Highest infection				Tree height			
	1	2	3	4	1	2	3	4	1	2	3	4
1990	4.4	4.7	4.4	5.4	6.1	5.9	5.8	6.3	11.0	10.5	10.9	12.6
1995	6.1	5.9	5.6	6.6	9.2	8.5	8.1	7.8	13.8	12.9	12.9	14.7
Increase	1.7	1.2	1.2	1.2	3.1	2.6	2.3	1.5	2.8	2.4	2.0	2.1

The mean increase in height between 1990 and 1995 is also given. The number of living trees in 1990 was 31, 24, 23 and 23 for survey line 1, 2, 3 and 4, respectively

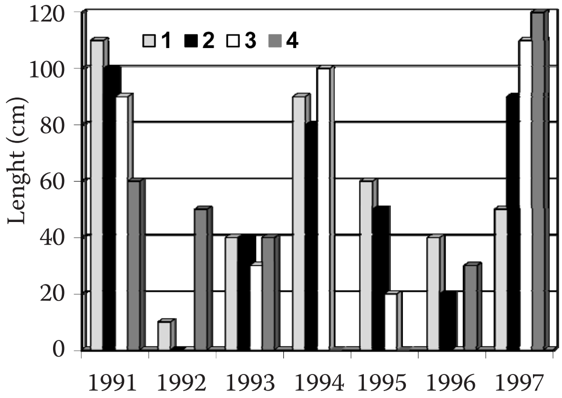

Fig. 3. Average annual infected length in cm per year measured from 1991 to 1997 on all trees of survey lines 1–4

Natural pruning, caused by the closure of the canopy and by the disease, continued to kill large branches. Based on measurements carried out from 1990 to 1995, the height of dead branches increased on average by 1.2 m, with the exception of survey line 1 where it was 1.7 m (Table 3). Average increase of the height of trees from 1990 to 1995 was greatest in line 1, even though in the end trees were taller in line 4 with an average of 14.7 m (Table 3).

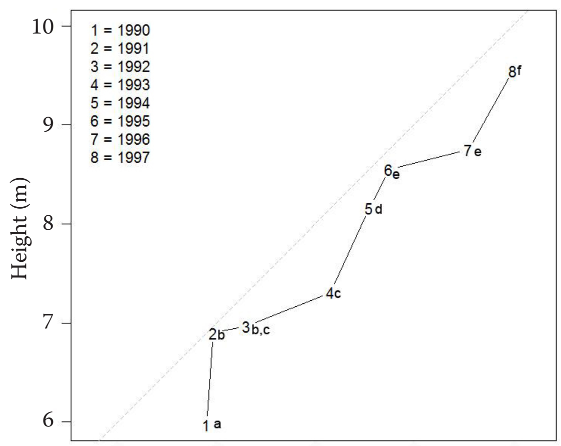

Fig. 4. Disease progression in site 1 from 1990 to 1997; the height (m) represents the average length from the ground where the disease has been observed. Years with different letters indicate significant differences at $P \geq 0.05$

In 1990 and 1995, the highest infection on average was recorded in line 1 and it was progressively found lower in the crown going through lines 2 to 4. The biggest difference between 1995 and 1990 was observed for line 1 with an increase in height of 3.1 m into the crown (Table 3). Data show an annual variation in the development of the disease in the crown of red pines (Fig. 3). In 1991, the new portion of the crown showing symptoms and signs of the disease was much higher in the four plots, ranging from 60 to 110 cm compared with 0 to 50 cm in 1992. A similar trend was measured in 1994 and 1997 when the disease seemed more severe. Actually, the disease always seemed to progress from 1990 to 1997, though significant differences from year to year were statistically significant only half of the time (Fig. 4).

Site 2

The length of the plantation measured from the road down to the river was 230 m and the difference in topography was 10 m between the water surface and the road (Fig. 5). All red pines located between the river and 90 m from it, i.e. the bottom part of the slope where the main portion of their crown was below the 10 m elevation line, were killed by *G. abietina* between 1991 and 1994, while pines on the upper part were diseased but still alive (Fig. 2). Before the epidemic, pines located either on the lower or on the higher elevation had the same height, with the exception of a portion between 40–90 m from the river. In the latter area, trees had slower growth and presented mineral deficiency symptoms.

Site 3

After 3 years, GaEU was found on 10 saplings planted in the field next to the La Macaza plantation. One seedling planted at the edge of the infect-

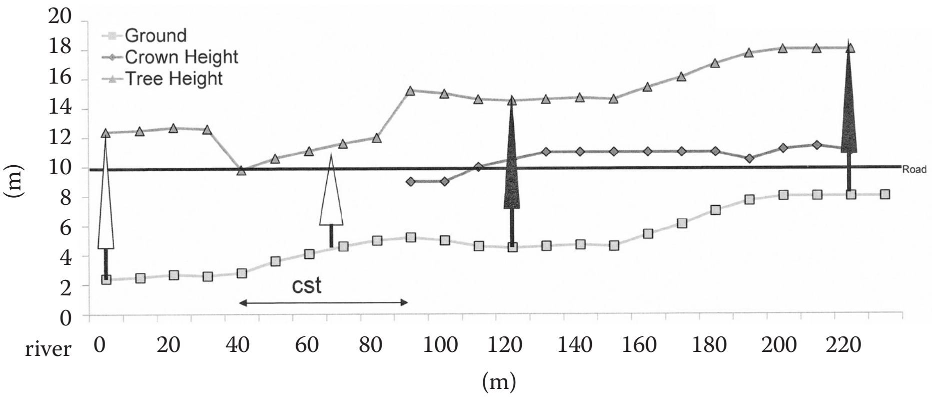

Fig. 5. Topography of the land at the red pine plantation located at Sainte-Anne-du-Lac (Site 2) measured in 2003, as well as the lower crown height and total height of trees. Between the river and 90 m, the total height of trees has been estimated from data collected in 1991: all these pines have been killed by *Gremmeniella abietina* from 1991 and 1994. Between 40 and 90 m, chlorotic and smaller trees (CST) died in 1991. The river is at 0 m and the road at 10 m

ed red pine plantation was killed by the pathogen while the other nine were within 20 m from the infected plantation. The number and the distribution of these seedlings were 2, 3, 1, 2 and 2 seedlings at 0, 5, 10, 15 and 20 m from the inoculum source, respectively. The disease did not spread further than 20 m away from the nearest infected red pines. The maximum distance of conidia dispersal was found to be a little more than the infection height of these pines. In fact, the average infection height of the pines in survey line 1 was 9.2 m in 1995, 5 years before the end of our experiments (Table 3). The average annual increase of infection in the crown of red pines in survey line 1 was 62 cm between 1990 and 1995; by extrapolation to the 1995–2000 period, the infected height in pines would be 12.3 m. From these data, it appears that conidia dispersal more or less follows a line extending at 60° from the height of the highest infected shoots.

DISCUSSION

The horizontal spread of *G. abietina*, European race (GaEU), is characterized by its relatively short distance of conidia dispersal. In site 3, in spite of a high load of inoculum produced by infected red pines on site 1 next to the experimental field, only seedlings located in the first 20 m from the plantation showed shoot blights over a 3-year period.

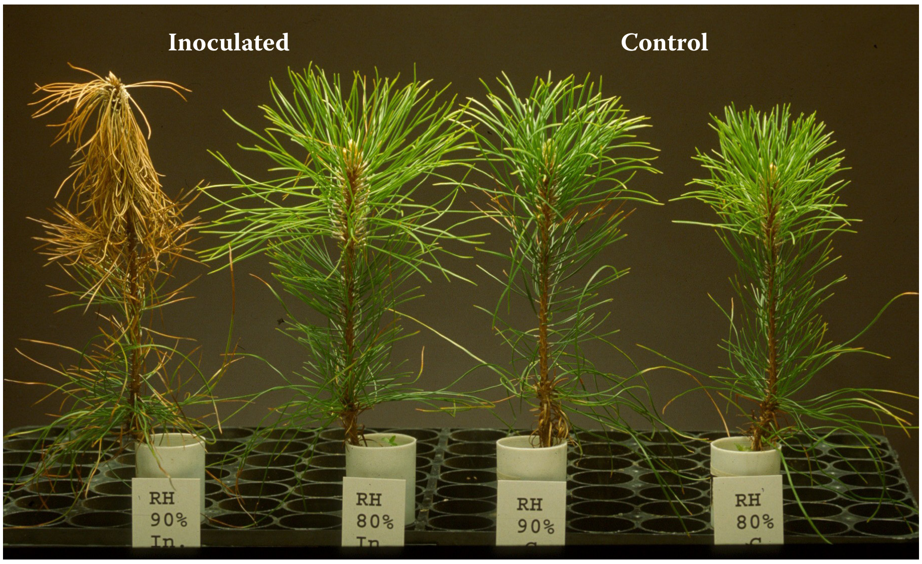

Fig. 6. Experiment with red pine seedlings, half inoculated with conidia of *Gremmeniella abietina*, European race, and the other half kept as control. One lot of seedlings was kept over 90% RH with free water on the foliage; the humidity was kept below 80% RH for the second lot. Temperature was between 0 and +1°C in a cold room. The duration of the experiment was 40 days

Skilling (1969) was able to trap ascospores during a 2-year study on *G. abietina*, North American race, but could trap conidia apparently only near the infected shoots; thus, he concluded that conidia are not disseminated by the wind but by rain splash. Skilling et al. (1986) were able to trap conidia (i.e. 1723) of GaEU at the edge of an infected site at Saranac Lake, New York State, USA. In five stations located 61 up to 610 m away from the same source of infection, very small numbers of conidia, i.e. around 20 per station, were trapped. These data show that the disease has the potential to spread through wind-borne conidia, though the probability that these few spores could cause any infection on pine appears very low. We believe that our method of using bioindicators to measure the distance of spore dispersal is much more valuable. In North America, for GaEU, the only known case of conidia dispersed by wind and causing an epidemic is in South Central Newfoundland (Warren et al. 2011). The disease first developed in a centre of infection on lower branches in the middle of the red pine plantation. As soon as the pathogen has reached the shoots at a height of about 2 m in the crown of pines, the wind and the cool wet conditions prevailing in that area surrounded by bogs contributed to the development of new infection points in the top of neighbouring pines. Two years later, the plantation was a total lost.

Propagation of the disease over long distances usually occurs through ascospore dispersal (Skilling et al. 1986). During our observations from 1990 to 1995, never did we observe any apothecia of GaEU, even though thousands of shoots were examined annually on several hundreds of red pines. We believe that the propagation of the disease over long distances mainly occurred through infected seedlings in North America (Laflamme 1999). In 1995 (Table 2), we noted that the percentage of infected trees was high, except in line 4 where this percentage had even decreased when one draws comparisons with the data in Table 1. However, these trees being older and taller than in the other survey lines (Table 3), the natural pruning caused by the closure of the canopy may have played a role in preventing the dissemination of the pathogen.

The mortality rate was relatively high in 1995 on trees of lines 1 and 2 with 19 and 21%, respectively (Table 2). In 1995, cankers were frequently observed only in line 1, where 71% of trees showed some damage (Table 2). Cankers are formed when the pathogen reaches the trunk while the trees are still alive. In line 1, in spite of heavy infestation, around 80% of the pines had survived (Table 2), still having a fair amount of green crowns (Table 3). The presence of cankers on the trunk seems to be a good indicator that the disease is spread at a slow rate towards the upper part of the crown.

When the epidemic of GaEU began in the early 1980s in eastern Canada, the mean age of red pines was 12 years old in plantations that were examined (Laflamme, Lachance 1987). Trees in plantations measuring less than 2–3 m in height were killed quite rapidly, while larger pines (i.e. 4 m and taller) were able to survive for several years. At the time, we realized that the spread of GaEU towards the top of the pines was an important parameter of the epidemic and the present study provides a better understanding of this phenomenon. In 1990, on site 1, the highest infections were about the same in all survey lines, varying from 5.8 to 6.3 (Table 3). However, in 1995, this height was greater in line 1 at 9.2 m than in the three other lines, where it declined at 8.5, 8.1 and 7.8, respectively, creating a more or less horizontal infection front line. On site 2, the infection line also appeared to be horizontal even though the ground clearly was a slope. Mortality of all trees was noted in 1991 at the bottom of the slope with shoot blights occurring at a height of over 4 m in the crown of those trees while all the trees located on the top of the hill remained alive in spite of infection on lower branches. Our data strongly suggest that conditions conducive to shoot infection below the horizontal line must be related to climatic conditions. The presence of fog or mist, causing deposition of free water on the foliage, likely plays an important role in disease development. Through artificial inoculations with conidia of GaEU (unpublished data), we successfully infected red pine seedlings by keeping free water on the foliage at 0°C to 1°C for 40 days. Seedlings inoculated in the same way but kept at a relative humidity below 80% did not display any symptoms (Fig. 6).

From our observations of GaEU in two different areas, it appears evident that conditions favourable to disease development follow a horizontal line at about 10 m over the lower elevation. Leaf wetness while trees are in dormancy appears to be an important climatic factor in the development of GaEU. Relative humidity and rainfall do not provide precise details about the occurrence of mist in the air. In a study involving *Gremmeniella balsamea* (Laflamme, Smerlis 2012), leaf wetness was the factor best correlated with ascospore dispersal (Laflamme, Archambault 1990). Models to estimate leaf wetness have been developed, but a review on this topic reported several problems (Huber, Gillespie 1992).

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