

# A Qualitative Assessment of Wind Dispersal of Resting Spores of *Synchytrium endobioticum*, the Causal Agent of Wart Disease of Potato

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## ABSTRACT

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*Synchytrium endobioticum*, the causal agent of potato wart disease, is soilborne. It is indigenous to home gardens throughout the island of Newfoundland. Scrapings from windows of a shed proximate to heavily infested soil yielded resting spores. To make a qualitative statement about wind dispersal, simple impaction devices were examined for periods between June and October. Spore recovery at the sample site during the sampling period was estimated at >1,000. The role of wind in dispersal, and its potential in the contamination of vehicles that exit the province, is discussed.

*Synchytrium endobioticum* (Schilberszky) Percival is recognized in Canada as a Schedule I quarantine soilborne pathogen (2). Its principal host is potato (*Solanum tuberosum* L.). In potato, it causes wart disease (alias black wart or canker), which induces galls on stem tissues (stem base, stolon bud, and tuber eye) (8). Also, it infects root tissues in tomato (*Lycopersicon esculentum* Mill.) and other Solanaceous species, but does not induce gall formation (7). Resting spores (Fig. 1) are produced in the pathogen's sexual cycle and infest the soil upon decay of the spore-bearing tissue. Gall masses vary in size from <1 g to >50 g fresh weight (9), the latter with a potential to release approximately 200,000 resting spores. Resting spores are capable of long-term survival ( $\pm$  40 years), tolerant of harsh environments in cold climates, and endogenously dormant (15,16). The pathogen is regarded as economically dangerous because of its easy dispersal by human activities (footwear, tools, vehicles) and its potential effect on the economy of eastern (mainland) Canada, were it to be introduced into that region (14). The protection of the eastern provinces and the northeastern United States from invasion by exotic pathogens such as *S. endobioticum* is the concern of quarantine authorities (3). Each

year, *S. endobioticum* resting spores are recovered from vehicles at the inspection stations at ferry-exit ports in Newfoundland (11) (Fig. 2).

The pathogen is indigenous to home garden soils in >94% of the populated regions of the island of Newfoundland (8). Jösting (13) observes that these "smallholders" optimize conditions for the pathogen through monoculture: infected tubers and crop debris left on soil surfaces after harvest, as well as potato peels on compost heaps, dry out and serve as centers of infection foci. He observed that fields distant from these inoculum sources become contaminated without the likelihood of modes of contamination other than that resting spores of the pathogen "be transported by wind or insects."

The apparent novelty of this mode of contamination by soilborne pathogens is dispelled in a recent review of current literature on wind dispersal of soilborne pathogens (4). In the northeast region of the Netherlands, potato nematode cysts are transported hundreds of meters during dust storms (J. Bakker, *personal communication*). In a survey of resting spore distribution in a monocultured potato field at Avondale, Newfoundland, samples of the top 5 cm of soil displayed an average >100 spores per gram of soil (M. C. Hampson, *unpublished*). Resting spores of *S. endobioticum* were found adhering to windows on a building downwind of this field. Therefore, a study was initiated to determine if resting spores of this soilborne pathogen can be disseminated by wind. If windborne, spores of the pathogen could contaminate vehicles, which might provide a means of long-distance transport of the spores.

## MATERIALS AND METHODS

**Location.** An agricultural substation at Avondale (Fig. 2) is located 133 m above

sea level and has a convex south-facing slope that is open to the southwest prevailing wind (10). The substation is accessed by a gravel road 4 km uphill and easterly from the fishing village of Avondale. The neighboring communities of Middle Arm and Conception Harbour to the north are separated from the substation by a line of hills and ridges, with the nearest small holdings approximately 2 km away in a valley <40 m above sea level. The substation was located in isolation to avoid contamination of other areas. The western part is used for testing potato cultivars for resistance to wart disease. The field is heavily infested with *S. endobioticum* and has been under potato monoculture since 1954. The field is divided into two sections, each measuring 33 × 20 m. Spore impact devices were set up alongside the lower section of the field.

**Impaction studies.** Impaction studies were carried out using microscope cover glasses, glass microscope slides, glass vials, and practice golf balls. The cover glasses, slides, and vials were set up and collected at different times, forming numbered series i to iii or i to iv.

For each cover glass, a no. 5 cork was impaled on the top end of a nail driven into the top of a 45-cm-long wooden stake. The proximal end of each cork was smeared lightly with household Vaseline, and an

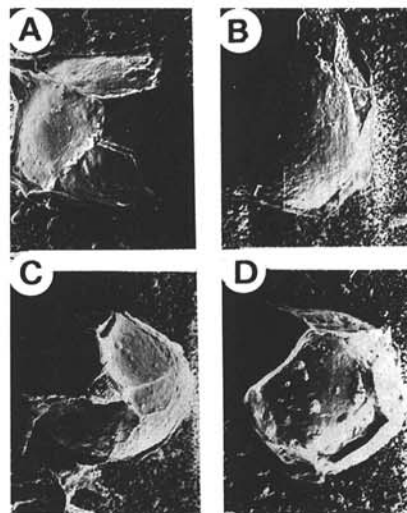


Fig. 1. Scanning electron micrographs of resting spores (average diameter, 60  $\mu$ m) of *Synchytrium endobioticum* showing ridges. Magnification: (A)  $\times$ 1,200, (B)  $\times$ 1,300, (C)  $\times$ 1,650, and (D)  $\times$ 1,750. (courtesy A. K. Bal)

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18-mm cover glass circle was pressed to each. The open face of the cover glass was smeared gently with a microthin layer of Vaseline. On 12 June 1995, 20 stakes were set approximately 1 m apart in a row parallel to and 2 m from the border potato row (drill) on the eastern side of the field. The cover glasses were about 30 cm above the soil, downwind from the prevailing wind. After 7 days (series i), the cover glasses were collected and taken to the laboratory. This procedure was repeated on 26 June and 11 July for 14 days (series ii) and 28 days (series iii), respectively. To accommodate a glass vial, a hole (25 mm diameter) was drilled vertically approximately 3

cm from the top in the side of a stake, and a 20-ml glass vial was inserted into the hole. On 11 July, 15 stakes were set 1 m apart on the eastern side of the field, vial-openings to the west. Five vials each were collected after 7 (series i), 14 (series ii), and 28 (series iii) days. A further set of nine vials was placed on the eastern side of the field for the period 14 to 20 September (series iv).

On 11 July, glass microscope slides were prepared and placed as follows. A thin coating of Vaseline was applied to a 20-mm circle at the center of a slide, and the slide was mounted on a split cork in a 1-liter open-ended milk carton in such a way

as to lie across the center of the carton to create a venturi-like entrapment surface. Each unit was mounted horizontally on one of the 45-cm-long stakes. The stakes were set on the eastern side of the field, and six, five, and one slides were collected on 17 July (series i), 24 July (series ii), and 8 August (series iii), respectively.

The practice golf balls were made of a plastic material (Par-Rite; KMC, Brampton, ON); the surface was smoothly finished and resistant to technical-grade chloroform. Outside and inside diameters were 4 and 3.8 cm, respectively, and bored with 18 equispaced 0.5-cm-diameter holes; impact surface area = 46.75 cm<sup>2</sup>. To produce an adhesive surface, a small quantity of household Vaseline was applied to the surface of a ball by hand. To secure the balls in the field, 45-cm-long stakes were topped with a 7.2-cm (3-inch) galvanized finishing nail. A rubber bung was added to secure the ball to the nail. Balls and stakes were set up approximately 3 m apart at the top (north) and bottom (south) sides of the field; balls 3 and 7 in both rows were Vaseline-free.

**Resting spore recovery.** Cover glasses or practice golf balls were immersed in technical grade chloroform, which freed the impacted rubbish for estimation of resting spore numbers. The resulting liquid was poured through a membrane filter circle. All filter circles were 47 mm diameter with 1.2- $\mu$ m pores. The matter was washed off into 100 ml of water, and 3  $\times$  10 ml aliquots were poured through additional gridded membrane filter circles. For each set of vials, the contents were flushed with water into a beaker, centrifuged, and decanted. The pellet was centrifuged with chloroform, and the spore component was floated off onto a gridded membrane filter circle. The matter was washed off into 100 ml of water, and the aliquots were examined for resting spores at  $\times$ 50. The Vaseline-smeared microscope slides were read directly at  $\times$ 50.

**Environment data collection and analyses.** Temperature ( $^{\circ}$ C), wind speed (km/h), and precipitation (mm) data were obtained from the Environment Canada meteorological station, St. John's Research Centre, Newfoundland. To register wind speed values at the station, wind speed data are accumulated continuously and recorded each 24 h. The plot data in Figure 3, therefore, are the quotients from dividing each recorded value by 24. Thus, the data represent only the average hourly wind speed per day. Temperature and precipitation data in Figure 3 represent the daily values at 1600 h LT and daily accumulations, respectively. These data were plotted using Microsoft Excel (version 3.8) software.

Gust data from the weather records at the Environment Canada meteorological station, Torbay Airport, St. John's, New-

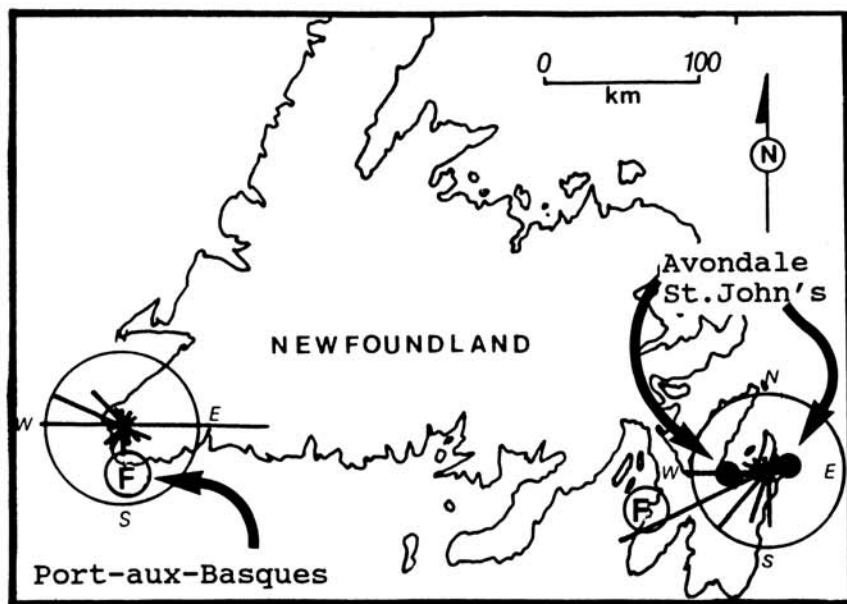


Fig. 2. Diagrammatic representation of island of Newfoundland showing location of ferry terminals (F) for embarkation to mainland Canada and wind direction frequencies in August for Port-aux-Basques and St. John's. The wind rose bars indicate direction (to center) and frequency of direction. The circle about each wind rose represents 12% wind frequency.

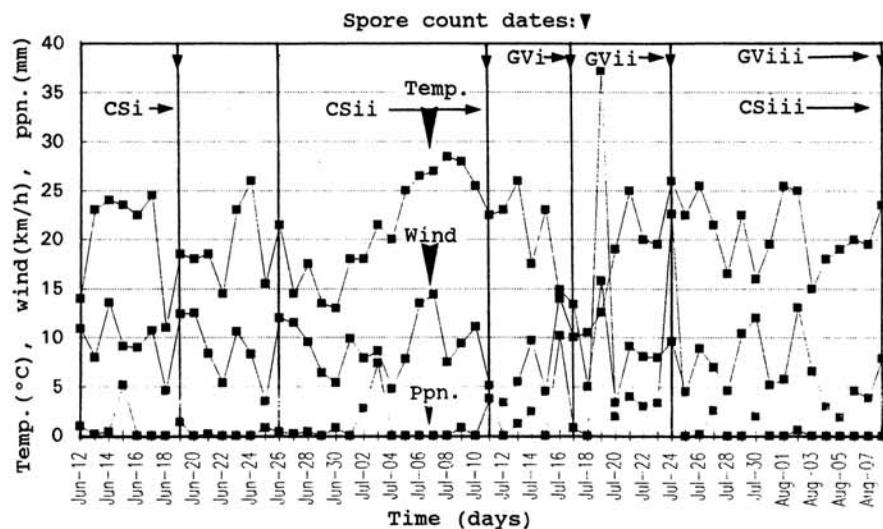


Fig. 3. Temperatures ( $^{\circ}$ C) at 1600 h LT, average hourly wind speed per day (km/h), and daily accumulations of precipitation (mm) recorded for the period 12 June to 8 August at the St. John's Research Centre. Each date on which Vaseline-smeared cover glasses (CS) and glass vials (GV) were collected for spore counts is shown as a perpendicular line + arrow.

foundland, for each impact collection period are presented in Table 1 in m/s.

Where impaction areas were measurable (glass circles, golf balls), a value termed relative impaction frequency (RIF) was determined:  $RIF = \text{no. spores/cm}^2/\text{day}$ . The total surface areas for each cover glass series and the golf balls were 50.9 cm<sup>2</sup> and 794.75 cm<sup>2</sup>, respectively.

## RESULTS AND DISCUSSION

The resting spore counts for the impact devices and their impaction periods, June to October, are presented in Table 1. Relative impaction frequencies were: (1) cover glass i, 1 spore/cm<sup>2</sup>/day; ii, 0.25 spore/cm<sup>2</sup>/day; iii, 0.016 spore/cm<sup>2</sup>/day; and (2) practice golf ball, 0.008 spore/cm<sup>2</sup>/day. The four Vaseline-free balls were treated as the control balls; it is assumed that the spores (five) recovered from them were from inside the ball.

The average daily wind speeds ranged between <1 and 4.1 m/s, only falling <1.4 m/s five times in the period 12 June to 8 August; 16 times in that period the speed was >2.8 m/s. The grand mean wind speed for the first observation period (of 58 days) was 2.3 m/s  $\pm$  <1.

Data collected in this study demonstrate that resting spores of *S. endobioticum* can be dispersed by wind. The simple morphology of the receptive surfaces facilitated the gathering of spore impact data. The practice golf ball presented both an omnidirectional impact surface and an entrapment volume in which air speed must fall in much the same manner as in pinecone scale-bract complexes (18).

In all, these simple collection devices yielded an estimated 1,143 resting spores. If the value of RIF for the initial set of cover glasses is approximately 1 spore/cm<sup>2</sup>/day, then theoretically, under similar conditions of wind (gusts) and temperature, a contiguous garden plot of 1 Are (= 100 m<sup>2</sup>) could receive 1,000,000 resting spores in a day, or a vehicle whose door panels and windows (=2 m<sup>2</sup>) present an impact surface downwind, could receive a daily dose of 20,000 resting spores. Gusts, however, are discontinuous; the discontinuities would modify these theoretical values. A quanti-

tative assessment of the effect of wind requires a complex and sophisticated treatment (19).

Jösting's (13) observation on resting spore dispersal of *S. endobioticum* in 1909 is the only reference in the literature on this disease or pathogen concerning the potential influence of wind. He relates dispersal to diseased potato material left to dry on soil surfaces after harvest. Campbell and Benson (4) emphasize the importance of operations associated with crop harvest that facilitate the dispersal of soilborne fungal pathogens by wind. Noteworthy was the detection of *Cylindrocladium crotonariae* in peanut root fragments 234 m downwind from a plantation (20) and *Verticillium albo-atrum* 6.1 m above ground in a potato-growing area (5). Most of the impacts observed in this study (cover glass, slide, and vial) were not related to harvest conditions, but to bare soil or soil in which crop cover was developing. At Avondale, planting takes place at about 1 June, so cover glasses (series i) were in place during the appearance of the first two to four leaves. Later collections occurred during the production of all the potato foliage. The practice golf balls were in place during harvest and were collected on 24 October.

Because of the simplicity of the impaction devices and the need only to qualify the phenomenon, correlations between spore numbers and weather patterns at this stage of investigation are difficult to state. The need to quantify the phenomenon is being pursued through the use of circular statistics (19).

A relationship is discernible between temperature and wind (Fig. 3). As the temperatures increased, so did the wind speeds. This suggests that a dry or a drying soil surface is allied to the potential for wind disturbance. The decreasing numbers of resting spores on cover glasses with time could reflect the increase in plant cover or an increase in precipitation (e.g., late July). The glass vial presents a variable impact surface, possibly related more to wind direction and speed than the cover glass. Several microscope slide assemblies disintegrated in severe gusts. The practice

golf ball presents an ideal omnidirectional surface.

Dispersal appears to be favored by a combination of fallow soil, periods of dry weather, warm air temperatures, constant wind flow, and the aerodynamic attributes of resting spores—notably the distinctive ridges (Fig. 1) that give it lift.

The data underline the common observation that "the wind always blows in Newfoundland" and exhibit all combinations of pressure, friction, stability, and squall (12). The "hurricane season" (from storms spawned off West Africa, via the eastern seaboard) in Newfoundland lasts from June to November (6) and encompasses the period of this study. The hourly gust data (Torbay Airport) show a high frequency of gusts >10.3 m/s. With a mean wind speed of >2.2 m/s (St. John's Research Centre), frequent gusts, and gust speeds ranging 7.5 to 30.5 m/s (Torbay Airport), the potential for airborne dispersal of *S. endobioticum* appears to be high. Wind velocities in the province are among the highest in Canada (32 to 37 m/s) (17). The mean wind speeds at St. John's (recorded at 140-m altitude) in July for the period 1955 to 1980 from N, NE, E, SE, S, SW, W, and NW were approximately 5.0, 3.9, 3.6, 4.4, 5.6, 6.6, 6.7, and 5.6 m/s, respectively (1). For the same month and period, the maximum hourly and gust speeds were 18.6 m/s (WSW) and 29.8 m/s (SSE), respectively, and the "all directions" wind speed was 5.9 m/s (1). Rowe et al. (20) recorded peanut root fragments 234 m downwind when wind speed was  $\leq$ 4 m/s.

No weather data were generated for the Avondale location per se. The location, however, (approximately 35 km WSW of the Research Centre) is close enough to standard meteorological stations for the values of dominant weather parameters to be pertinent. The Avondale field is subject to the same general weather components as the cited meteorological stations, and although local variations in airflow patterns (6,12,17) may have some effect on the degree of dispersal, it is clear that wind dispersal of *S. endobioticum* is possible.

**Table 1.** Spore counts of *Synchytrium endobioticum* and wind gust velocities. The spores were collected from various impaction surfaces arranged contiguously with an infested potato field and exposed for different periods of time throughout the growing season

Type	Impact device		Date	No. days	Max. daily gust, m/s		Spore count
	Series	No.			Range	Mean $\pm$ SE	
Cover glass	i	20	06/12-19	8	13.9-20.0	17.1 $\pm$ 1	280
	ii	20	06/26-07/9	14	10.3-20.6	16.7 $\pm$ 1	180
	iii	17	07/11-08/7	28	9.2-18.1	13.7 $\pm$ <1	23
Glass vial	i	5	07/11-17	7	9.2-17.5	13.7 $\pm$ <2	40
	ii	5	07/11-24	14	9.2-18.1	14.3 $\pm$ >1	200
	iii	5	07/11-08/7	28	9.2-18.1	13.7 $\pm$ <1	120
	iv	9	09/14-20	7	12.3-24.1	18.5 $\pm$ >2	45
Glass slide	i	6	07/11-17	7	9.2-17.5	13.7 $\pm$ <2	1
	ii	5	07/11-24	14	9.2-18.1	14.3 $\pm$ >1	0
	iii	1	07/11-08/7	28	9.2-18.1	13.7 $\pm$ <1	1
Golf ball		17	09/14-10/24	41	9.6-30.5	16.6 $\pm$ <1	255

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