Potential Spread of Erwinia spp. in Aerosols

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ABSTRACT

Bacterial aerosols were generated in a chamber by simulated raindrops falling on potato stems infected with *Erwinia carotovora* var. *atroseptica*. Viable propagules moved readily with slow air streams and remained suspended in the air for at least 60-90 minutes. Phytopathology 65:739-741

Additional key words: dissemination, sprinkler irrigation.

Soft rots of vegetables and other plants caused by *Erwinia* spp. are important problems everywhere. The pathogens are reported to be soil-borne, and contamination of fleshy organs directly from the soil has been believed to be the primary source of inoculum. Newer evidence, however, indicates that *Erwinia* spp. do not survive well in soil (5, 8,), at least in temperate climates. Potatoes freed from *Erwinia* have become recontaminated in isolated areas (6), and other crops not vegetatively propagated are often infected. This raises important questions about inoculum sources. Splash dispersal may spread bacteria and wind-blown rain has been implicated in the spread of some bacterial pathogens for longer distances (4). The possibility exists that aerosols generated by rain or overhead sprinklers could be responsible for long-distance spread of soft-rotting bacteria. Southey and Harper (9) reported the survival of artificially generated *Erwinia amylovora* aerosols for 3 hours, but no other known information on the occurrence and potential importance of aerosols as a means of spread has been reported for bacterial plant pathogens. This report describes laboratory experiments to determine if aerosols can be generated from potato tissues infected with *Erwinia carotovora* var. *atroseptica* (van Hall) Dye by impaction of water drops.

MATERIALS AND METHODS.—Water drops ranging from 2 mm to 5 mm in diameter were dropped down a 15.24-cm diameter plastic tube from a height of 7.62 m onto infected potato stems (cultivar Pentland Javelin) placed in a flat tray (containing approximately 3.81 cm of sterile soil) located in a stainless steel tank large enough to contain any splash droplets (Fig. 1). Water drops 2 mm in diameter falling from 7.62 m reach terminal velocity while 5 mm drops reach 94% of terminal velocity (7). Humidified air (approximately 90-95% RH and 10.6 C) was drawn into the tank then out through a wind tunnel made of 30.5-cm diameter aluminum tubing 2.74 m long by a variable-speed fan located at the exit end of the tube. Ports located at 30.5-cm intervals along the tube facilitated sampling the moving air. The distance from the target site to the end of the tunnel was approximately 4.1 m.

A Casella bacteria slit sampler (C. F. Casella and Co. Ltd., London, England) connected with a rubber tube to the sampling ports was used to sample air moving through the tube at various distances from the target stems. Bacteria were deposited on Stewart's double-layer pectate medium (10) in petri dishes which were subsequently incubated at 26 C for 48 hours, then examined for *Erwinia* colonies. Pectolytic *Erwinia* spp. form characteristic colonies on this medium which can be readily distinguished from other pectolytic bacteria such as *Pseudomonas* spp., *Bacillus* spp., and *Flavobacterium* spp. which may be associated with potato stems.

RESULTS.—*Generation of aerosols by simulated raindrops.*—Water drops 5 mm in diameter fell on six stem sections approximately 15.2 cm long at the rate of 960 drops per minute for 5 minutes. Air was drawn through the system at the rate of approximately 22.9 m/minute. The air was sampled approximately 4.6 m from the stems at the volume of 30 liters per minute for 10-minute periods beginning at the time drops began to fall, and ending 15 minutes after drop delivery had ceased. The system was checked for contamination by following the above procedure using healthy stems immediately before the experiment with infected material.

Large numbers (approximately 600 colonies per plate) of airborne *Erwinia* were collected during the first 10 minutes of the experiment and some (four colonies per plate) were collected for at least 10 minutes after water drops had ceased. This suggests that bacterial aerosols that move with a slow air stream were generated by the simulated raindrops. The experiment was repeated using a slower rate of simulated rainfall (130 drops per minute) with similar results.

Relation of raindrop size and number to aerosol generation.—Since 5 mm water drops approach the maximum size of raindrops (1) an experiment was designed to study the effect of drop size and number on aerosol generation. Drops 2, 3, 4, and 5 mm in diameter bombarded infected potato stems with the air flow and humidification systems shut off. Immediately after the

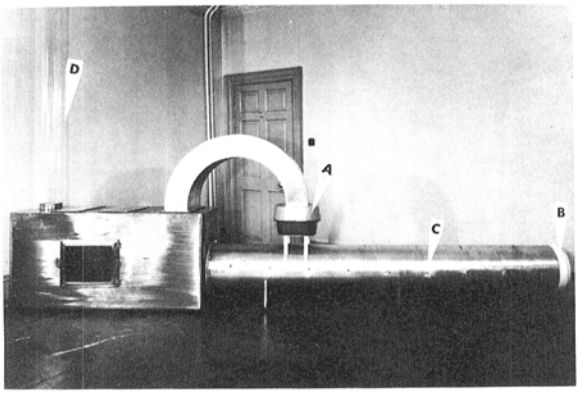

Fig. 1. Apparatus used to study bacterial (*Erwinia carotovora* var. *atroseptica*) aerosol formation by simulated raindrops striking infected potato stems. Legend: A=humidifier, B=variable-speed fan, C=port for sampling air stream, D=plastic tube through which water drops were delivered. Dimensions of the main chamber were: height 76.2 cm, width 55.9 cm, and length 152.4 cm.

TABLE 1. Relation between size and number of simulated raindrops and generation of bacterial (*Erwinia* sp.) aerosols from blackleg-infected potato stems

Drop size (mm)		Number of drops	Number of *Erwinia* colonies/150 liters of air[a]
2		1	0
		5	1
		10	2
		25	8
		50	7
		100	17
	Total		35
3		1	1
		5	15
		10	0
		25	34
		50	33
		100	57
	Total		140
4		1	32
		5	0
		10	18
		25	42
		50	84
		100	104
	Total		280
5		1	43
		5	30
		10	49
		25	53
		50	32
		100	49
	Total		256

[a]Air was sampled at the rate of 30 liters/minute for 5 minutes at a point approximately 4.0 m from the target stems. Air sampling began immediately after the designated number of drops had struck the diseased stems.

required number of drops had struck the stems, the systems were turned on and air (92% RH) was drawn through the apparatus at 18.3-21.3 m per minute. Air was sampled for 5 minutes (150 liters of air sampled) at a point approximately 4.0 m away from the target area. Control plates were exposed between treatments, and plate counts were adjusted to compensate for any bacteria remaining in the system between treatments.

The results (Table 1) show that detectable numbers of bacteria became airborne after the stems were struck by as few as five drops 2 mm in diameter or one drop 3 mm in diameter. Bacterial numbers increased as drop size and number increased up to 4 mm. There was no further increase in bacterial numbers using drops 5 mm in diameter.

Suspension time of bacterial aerosols.—An experiment was designed to determine the length of time aerosols generated by water drops remained suspended in the system. Infected potato stems were bombarded with 5 mm diameter water drops falling at the rate of 1,000 drops per minute for 5 minutes. During this time the air flow and humidification systems were turned off, but immediately after the simulated rainfall ceased, the fan and humidifier were started and air was pulled through the system at a velocity of 21-22.9 m per minute. The air was sampled for a period of 30 seconds at a point approximately 4.0 m from the target area then the air flow was immediately stopped. The process was repeated at 15, 30, and 60 minutes after the rainfall ceased. At 90, 120, and 150 minutes the air was sampled for 2 minutes and at 180 minutes the air was sampled for 5 minutes.

Detectable numbers of viable particles remained suspended in the system for at least 60 minutes after simulated rainfall ceased. Bacterial numbers decreased from approximately 300-400 colonies per plate in the initial sample to 1 colony per plate in the 60-minute sample. No bacteria were detected in the 90-, 120-, 150-, and 180-minute air samples.

The actual number of aerosol particles containing bacteria per unit volume of air could not be accurately determined since sampling was anisokinetic; i.e., the air speed in the sampling tube was much greater than in the wind tunnel.

DISCUSSION.—The formation of airborne droplets containing viable bacteria which remain suspended for a relatively long period of time and move in slow air streams adds a new dimension to the epidemiology of soft rots and related diseases caused by *Erwinia carotovora* var. *atroseptica* and similar organisms. Aerosols generated by rain showers or sprinkler irrigation systems in arid areas may move considerable distances on light breezes under the appropriate conditions. Because some

particles remained suspended in still air for 60 – 90 minutes they must be small; i.e., in the 4- to 8-μm range (3). Once airborne, propagules of this size may remain suspended for prolonged periods in moving air.

Formation of *Erwinia* aerosols in this same size range probably occurs in the field as indicated by limited sampling in Colorado.

The susceptibility of *Erwinia* cells to desiccation, radiation, open-air factor (2), etc. will determine the potential role of aerosols in the epidemiology of soft rot bacteria, but nothing is yet known about these aspects.

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