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Monolayers and Microbial Dispersal

Abstract. *Aqueous films on terrestrial litter are inhabited by numerous microorganisms; the surfaces of such films are covered by monolayer-forming substances. The spreading pressure of the latter can result in transport of floating and submerged organisms to adjacent water films with clean surfaces. The clean surfaces, produced by rain or possibly dew, permit rapid vertical and horizontal dispersal of microorganisms onto newly fallen leaves and other plant materials.*

During wet periods, a thin layer of water is present on leaves and other litter on the soil surface. This film is the habitat of numerous bacteria, protozoans, fungi, and other small organisms (*1*). The organisms inhabiting the film can be seen readily if moist litter is submerged in water in a glass dish and the air-water interface is examined microscopically (*2*). Among the more conspicuous objects often present are floating propagules (conidia) of various Fungi Imperfecti. While examining such conidia on water, we saw that the surface layer of water and floating objects often moved opposite the flow of the underlying water if the container was tilted. Furthermore, adjacent objects at the interface maintained a constant position with respect to one another, which suggests that the presence of monolayer substances at the air-water interface was responsible for the movement observed. These observations led to a series of experiments that demonstrated a previously undescribed mechanism for local dispersal of terrestrial microorganisms.

Initially, we used conidia from cultures of *Articulospora tetracladia, Cladosporium* sp., *Gyoerffyella (Ingoldia) craginiformis, Fusarium* sp., *Penicillium* sp., and *Varicosporium elodeae*, all of which had been isolated from the surface film from litter. Because of the masses of spores available, basidiospores of a puffball, *Lycoperdon perlatum*, spores of a myxomycete, *Fuligo septica*, and cells of bakers' yeast, *Saccharomyces cerevisiae*, were used in later experiments. With the exception of the yeast cells, all of these propagules float, either because of their form or because of hydrophobic surface layers. Masses of the spores were floated onto the surface of tap water in reservoirs (glass dishes). Clean glass strips 5.0 cm wide, 0.6 cm thick, and 1 m long were used to form inclined planes (Fig. 1), the lower ends of which terminated in the reservoirs with floating spores. In some experiments, the glass strips were used alone; in others, the strips served as support for either dialysis tubing or leaves. Distilled water (glass, distilled once) was allowed to drip slowly onto the upper end of the glass strip and to flow down the incline to the reservoir. In tests involving leaves, only senescent or recently fallen leaves of *Acer macrophyllum* were used; they were arranged shinglewise on the strip. Dialysis tubing was first wetted, then slipped over the glass strips and smoothed.

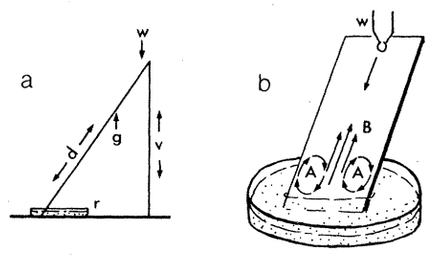

Fig. 1. (a) Diagram of experimental apparatus; *r*, reservoir with water and spores; *g*, glass strip; *w*, point of water application; *d*, distance from source traveled by spores; *v*, vertical distance traveled by spores. (b) Diagram showing surface motion of spores on water; *A*, rapid circulating motion; *B*, slow or rapid movement of spores toward water source at *w*.

As water flowed down the inclines, three different types of movement of the spores were observed: (i) rapid circulating movement from the reservoir surface onto the incline and back to the reservoir (*A* in Fig. 1); (ii) slow creeping movement of the spores up the wet surface of the incline (*B* in Fig. 1); and (iii) rapid movement of the spores onto the surface of the incline. These movements were, in part, correlated with rates of flow of water down the strip. The rapid circulating movements accompanied a high rate of flow; slow spread occurred while a very slow flow of water was maintained or after such a flow had been stopped; rapid spread occurred immediately after rapid flow was cut off. The greatest distances traveled by the spores were obtained when a very slow flow was maintained and then stopped. The distances are indicated in Table 1.

In the experiments described here, spores were transported equally well on clean glass strips, on dialysis membrane on such strips, and on leaf surfaces. No differences could be detected in distance of transport of differently shaped propagules; suspended yeast cells were transported to the same distances as floating propagules. When leaves were used, water was directed on the upper edge of the leaf and flow occurred mainly along the vein patterns. Both single leaves and laminations of several overlapping leaves were tested. The edges of laminated leaves did not present an insurmountable barrier to the upward transport of spores. However, it was found that living leaves, or such leaves pressed and dried, did not work well in these experiments. The intact cuticle prevented wetting of the surface.

Spores of *Fuligo* and *Lycoperdon* and yeast cells were used in large quantities; their transport could be followed visually. The distances traveled by other propagules used in the tests were determined by pressing microscope cover slips to the wet surfaces of leaves, then examining them microscopically. When glass strips alone or strips with dialysis membrane were used, the films were allowed to dry and the surfaces were then scanned with a microscope to determine the distances traveled.

In a second type of experiment, clean glass microscope slides or washed

leaves were wetted with distilled water, then lowered vertically into reservoirs containing water and floating propagules. In these tests, the propagules often appeared to explode onto the surface of the leaf or slide.

In a third set of experiments, techniques employed by rheologists were used (*3–5*). Precautions were taken to obtain very clean distilled water (*5*) and all glassware was cleansed. Paraffin-coated reservoirs were substituted for glass dishes and purified talc replaced the floating fungus propagules. The surface of the distilled water (glass, distilled three times) was swept to remove any contaminating films. The apparatus and tests were as described above, but leaves and membranes were omitted. In these tests, movement of the talc was either nil or slight; where slight movement occurred, it was attributed to surface impurities on the water. Addition of a drop of either corn oil or oleic acid to the reservoir water resulted in rapid transport of floating talc onto inclined or vertically held glass strips. We did not attempt to determine either the maximum vertical distances or the speed of such transport. We did observe vertical transport to a height of 17 cm.

Our laboratory experiments demonstrated the feasibility of microbial dispersal by spreading films at the air-water interface. To test whether monolayer substances in nature had similar effects, two types of experiments were performed. First, samples of pond and aquarium water were brought into the laboratory and used in reservoirs for experiments essentially like those described previously. Talc was used to indicate movement and to determine whether films were present. Substances present on the surfaces of these water samples drove the talc up angled or vertical glass strips. When the surfaces of such water samples were first swept clean of monolayer substances and then tested, no movement of the talc occurred.

We then placed acid-cleaned microscope slides in containers of freshly prepared distilled water and took them to nearby ponds. The wet slides were held vertically and lowered until the lower end contacted the water surface. A small area of the surface had been sprinkled previously with talc to allow visual detection of any motion. Vertical movement occurred, as in the laboratory experiments, and cover slips were applied to the upper edge of the flow as indicated by the talc. Similar tests were made with the wet films on leaves adjacent to, or at some distance from, the ponds. The slides were then examined microscopically and all showed a variety of microorganisms, including algae, protozoans, bacteria, and fungal spores.

Table 1. Maximum observed distances traveled by spores: *d*, distance from source; *v*, vertical distance (see Fig. 1). The angle is that between the water surface and the glass strip.

Angle	*d* (cm)	*v* (cm)
15°	27.5	7.2
30°	28.0	15.1
45°	30.0	19.1
90°	22.5	22.5

In 1773, Benjamin Franklin (*6*) observed that spreading oil films moved leaves and other debris on the surface of Clapham Pond in England. In later experiments, Reynolds (*7*) also noted that dust on the surface of water was pushed ahead of advancing oil films. Adam (*8*) first showed that monolayer-forming substances were present on the surfaces of ponds, rivers, and other water bodies. Adam, and later Goldacre (*9*), used oils of known spreading pressure to measure the spreading pressure of such naturally occurring films. Goldacre determined that film-forming substances were produced by decaying leaves, mold spores, and other organic matter.

Abribat *et al.* (*10*) and La Mer and Blank (*11, 12*) performed experiments involving the separation of mixed monolayer-forming substances. Their apparatus consisted of two reservoirs, interconnected by an arch. Movement of the substances occurred on the surface of water in a groove on the upper surface of the arch, demonstrating some vertical spread of films. La Mer and Blank (*11, 12*) observed that transfer of films occurred, even when the receiving reservoir was higher than the source reservoir, that is, that surface flow occurred against a hydrostatic head maintaining a bulk flow of water in the opposite direction to the spread of the film. It had been shown by Schulman and Teorell (*13*) that a monolayer moving over water carries with it a considerable volume of water. An apparent boundary layer with a thickness of about 0.03 mm was determined; the thickness was dependent on the viscosity of the substrate but independent of the velocity of the monolayer (*13*).

Muggoch and Walton (*14*) observed rapid horizontal spread of moss spermatocytes at the air-water interface upon dehiscence of antheridia. They also observed that *Lycopodium* spores dusted on the water surface were swept ahead of the spreading spermatocytes, and suggested that spreading was caused by fatty substances in the antheridia. Such substances could conceivably push the spermatocytes vertically along the culms of these plants as well. Amies (*15*) studied populations of bacteria and their horizontal dispersal at the air-water interface of swimming pools. He attributed dispersal to monomolecular substances such as proteins in saliva and lipids from skin.

Most experiments by rheologists have involved horizontal spread of films. Our experiments demonstrated both lateral and vertical transport of microbial structures; transport occurred whether the structures floated or were submerged. Presumably, submerged cells were transported only if they were within the narrow boundary layer dragged by the spreading monolayer substances. The movements observed are more than ample to cover newly fallen leaves with decay organisms or their propagules. Wet, falling leaves could be covered almost instantaneously by a layer of spores, bacteria, and so forth, at the moment of contact with the litter layer. Alternatively, rain falling on the leaf carpet and flowing down leaves to the underlying film must result in spread of monolayer substances onto the overlying leaves with concurrent transport of microorganisms. Heavy dew conceivably could replace rain in producing the clean water surfaces required in this type of dispersal.

The mechanism we have described must ensure contact of decay organisms with each leaf in the carpet, either as deposited or whenever precipitation occurs. This assumes that litter at the soil surface serves as a reservoir for leaf decay fungi and associated organisms. Alternative possibilities, such as aerial dispersal, cannot be as effective, especially if the leaf layer is formed rapidly.

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Host-Specific Phytotoxic Polysaccharide from Apple Tissue Infected by Erwinia amylovora

Abstract. *A toxin isolated from apple fruit tissue infected by* Erwinia amylovora *is 98 percent galactose in polymeric form, 0.375 percent protein, and has an average molecular weight of approximately 165,000. Young shoots of rosaceous, but not nonrosaceous, species wilt in a manner characteristic of the disease when placed in toxin solutions with concentrations as low as 10 micrograms per milliliter. Varieties of apple and pear susceptible to* Erwinia amylovora *wilt in 1 to 3 hours, whereas resistant varieties display symptoms 12 to 24 hours after treatment.*

The enterobacterium *Erwinia amylovora* (Burrill) Winslow *et al.* is pathogenic to many plant species of Rosaceae (*1*); however, it is most important economically on apple and pear. It is responsible for limiting commercial cultivation of favored susceptible pear varieties—for example, limiting Bartlett to the northwestern United States—and has annually reduced apple and pear crops in the United States and elsewhere (*2*).

The biochemical basis for pathogenesis has received significant attention (*3*); however, the precise manner by which host cell damage occurs has remained unknown. Degradation of host cell walls by cellulases and pectinases has been suspected but has never been established, and protease production by the pathogen in vitro (*4*) has not been causally linked with pathogenesis.

Many researchers have sought to relate pathogenicity to toxigenicity, and this too has not yielded positive results (*5*). Nevertheless, Hildebrand (*6*) did report that a component of the bacterial "ooze," which exudes from infected tissue, could reproduce a part of the disease syndrome. He observed that pear shoots wilted within 2 hours when their cut ends were immersed in sterile ooze diluted to 50 times its volume. The ooze or bacterial capsule has also been shown to maintain viability of the pathogen under adverse conditions (*7*).

We report here the nature of the toxigenic agent and its relationship to pathogenesis in the disease called fire blight caused by *E. amylovora*.

A bacterial suspension of a virulent strain of *E. amylovora*, at a concentration of 2×10^9 cells per milliliter, was prepared from a 24-hour culture grown on nutrient agar fortified with 0.5 percent yeast extract and 1 percent glucose. One-half milliliter of the suspension was pipetted onto the surface of slices of aseptically sectioned green Jonathan apple fruits. Inoculated apple slices were kept in a moist chamber at 28°C for 3 to 4 days. Bacteria-laden ooze produced on the surface of inoculated apple slices was collected by capillary pipette and diluted to 50 times its volume with distilled water. The diluted ooze solution was centrifuged at 20,000*g* three times for 30 minutes, and the supernatant fluid was filtered through a 0.45-μm Millipore membrane. Characteristically, the filtered ooze solution has a *p*H of 7.45 and 2.38 mg of dry matter per milliliter. One milligram of the dry matter contains 0.966 mg of carbohydrate, 0.012 mg of amino acids, and traces of protein and lipid (*8, 9*). The polysaccharide-containing solution was passed through columns of Dowex 1 (Cl^- form, 2.5 by 25 cm) and Dowex 50 (H^+ form, 2.5 by 15 cm). The effluent was evaporated under reduced pressure to one-tenth the original volume. Polysaccharide was precipitated with three volumes of 95 percent ethyl alcohol, washed with absolute ethyl alcohol, dried under vacuum, and finally dissolved in a small amount of water and lyophilized.

One milligram of the lyophilized

Table 1. Specific activities of the phytotoxic polysaccharide against rosaceous species. The activity is expressed as the length of time in hours required for a plant cutting to show wilting when its base is placed in toxin solution at a concentration of 100 μg/ml. The following nonrosaceous species were exposed to toxin solution at 500 μg/ml for 24 hours and showed no wilting: *Lonicera tartarica, Forsythia suspensa, Medicago sativa, Melilotus altissima, Lespedeza stipulaceae*, and *Lycopersicum esculentum*.

Plant tested	Specific activity (hours)	Susceptibility to *Erwinia amylovora*	References
Pyrus malus			
Jonathan	1	Susceptible	(*17*)
Malling-26	1	Susceptible	(*18*)
Red Delicious	18	Resistant	(*19*)
Pyrus communis			
Bartlett	2	Susceptible	(*17, 20, 21*)
Starkrimson	3	Susceptible	(*19*)
Magness	12	Resistant	(*21*)
Moonglow	12	Resistant	(*21*)
Kieffer	12	Resistant	(*17, 20, 21*)
Starking Delicious	14	Resistant	(*20*)
Old Home	15	Resistant	(*20*)
Cydonia oblonga			
EM-A	8	Moderately resistant	(*19*)
Province	12	Resistant	(*19*)
Polish 1	12	Resistant	(*19*)
Polish 2	24	Very resistant	(*19*)
Polish 3	18	Resistant	(*19*)
Crataegus crusgalli	1	Susceptible	(*22*)
Spiraea corymbosa	1	Susceptible	(*22*)
Cotoneaster pyracantha	6	Susceptible	(*22*)
Sorbus americana	6	Susceptible	(*22*)