

COLONIZATION OF AUTUMN-SHED LEAVES BY FOUR AERO-AQUATIC FUNGI

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ABSTRACT

Colonization of freshly-fallen leaves by buoyant propagules of aero-aquatic fungi was demonstrated by introducing *Acer saccharum* leaves onto the surface of a small heterotrophic woodland pond. Subsequent degree of colonization by *Hormiactis ontariensis*, *Beverwykella pulmonaria*, *Pseudoaegerita matsushimae* and *Helicodendron trilitziense* was significantly correlated with duration of stay on the water surface. The substrate preferences of these fungi were also investigated. *Pseudoaegerita matsushimae* and *H. ontariensis* sporulated more frequently on freshly-abscised, than on year-old *A. saccharum* and *Fagus grandifolia* leaves. *Beverwykella pulmonaria* and *H. trilitziense* sporulated more frequently on year-old *Acer saccharum* leaves than on freshly-abscised leaves of the same species. *P. matsushimae*, *H. ontariensis* and *H. trilitziense* all sporulated more frequently on freshly-abscised than on year-old *F. grandifolia* leaves while *B. pulmonaria* exhibited no age preference.

Key Words: Aero-aquatic fungi, *Hormiactis ontariensis*, *Beverwykella pulmonaria*, *Pseudoaegerita matsushimae*, *Helicodendron trilitziense*, *Fagus*, *Acer*, lentic systems, ecology

In heterotrophic aquatic systems which depend mainly on fallen leaves to meet their energy needs, the primary leaf conditioners are aquatic ascomycetous or basidiomycetous anamorphs (Baerlocher and Kendrick, 1974). In small lentic systems, e.g., woodland ponds, aero-aquatic fungi predominate (Michaelides, 1982). Like other aquatic anamorphs, these grow in submerged detritus, e.g., leaves or twigs. However, their propagules are produced above the surface, in air, rather than below it. The propagules are unique, buoyant structures which float because they entrap air during development (Michaelides, 1982; Michaelides and Kendrick, 1982). At maturity aero-aquatic propagules become detached and float about freely on the water surface. Here, they are ideally situated to be the first aquatic fungi, and perhaps even the first aquatic organisms, to colonize leaves as they enter the pond.

We postulate that riparian leaves falling into a lentic system where aero-aquatic fungi predominate are quickly colonized by their floating propagules. Colonization at the air-water interface may not be the only means by which these fungi infect new leaves but it is, in our opinion, the primary one, as these fungi produce only buoyant propagules. Submerged leaves and twigs can also be colonized, presumably by vegetatively

spreading mycelia. However, this is a slow process requiring up to a month (Fisher, 1979).

MATERIALS AND METHODS

Fieldwork was carried out at a small woodland pond in the Laurel Creek Conservation Area (Waterloo, Ontario). The pond is heavily shaded by *Acer saccharum* Marsh, *Fagus grandifolia* Ehrh, *Fraxinus americana* Marsh and *Tsuga canadensis* Carriere, whose branches overhang the pond. *Acer saccharum* and *F. grandifolia* predominate and the pond receives large inputs of leaves from these trees. In summer the pond is almost fully shaded by the trees which grow around and over it, and this apparently limits primary production. At least 75% of leaves entering this pond are derived from *A. saccharum* (Michaelides, 1982).

Colonization of leaves.—These experiments were carried out in mid-autumn. One hundred freshly fallen maple leaves were attached, at regular intervals, to thin, light threads. These were lowered onto the water surface and the leaves allowed to float for 5 min, 6 h or 24 h. Controls ($t = 0$) were not exposed to the water surface. No leaves sank during the 5-min or 6-h exposures. However, after 24 h the majority of leaves had become waterlogged and were submerged. After the appropriate period leaves were lifted vertically from the water with a minimum of disturbance, and

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TABLE I
PERCENT OF *ACER SACCHARUM* LEAVES COLONIZED BY AERO-AQUATIC FUNGI FROM PROPAGULES
ACQUIRED ON THE WATER SURFACE^a

Exposure times	Total		<i>Pseudoaegerita matsushimae</i>		<i>Helicodendron triglitzense</i>
	Present ^b	Sporulating ^c	Present	Sporulating	Present
0	0	0			
5 min	92.84 ± 3.60	79.99 ± 5.86	81.50 ± 0.93	69.70 ± 1.48	13.80 ± 4.35
6 h	92.37 ± 4.65	85.67 ± 15.00	85.88 ± 1.72	80.50 ± 0.98	34.34 ± 0.65
24 h	91.84 ± 0.32	90.55 ± 0.68	83.00 ± 0.009	77.77 ± 4.38	53.50 ± 0.98

^a Means of 3 replicates of 100 leaves ±95% confidence interval.

^b Present: aero-aquatic propagules present on *Acer saccharum* leaves after exposure to water surface for t = 0, 5 min, 6 h or 24 h.

^c Sporulating: aero-aquatic propagules sporulating from leaf after incubation in a damp chamber at 10 C for 14 days.

were immediately placed in damp chambers, Petri plates lined with moist Whatman #1 filter paper, with the side previously exposed to the water surface now facing up. Each experiment was carried out three times.

Leaves sampled in this manner were examined under a dissecting microscope using fiber optic-transmitted light to prevent possible heat damage to any propagules that were present. The number of leaves with aero-aquatic propagules attached and the species to which these belonged were noted. *Hormiactis ontariensis* Matsushima, *Beverwykella pulmonaria* (Van Beverwijk) Tubaki, *Pseudoaegerita matsushimae* (Matsushima) Webster, and *Helicodendron triglitzense* (Jaap) Linder, were the only species found on the leaves. Leaves were then incubated at 10 C and examined twice a week to determine whether colonization had been successful. A leaf was determined to have been successfully colonized if new propagules developed on its surface following incubation at 10 C for 14 days (Fisher, 1979). The number of species sporulating on each leaf was recorded.

Substrate preferences of aero-aquatic fungi.—Submerged *A. saccharum* and *F. grandifolia* leaves were sampled twice during the year. The first samples were taken in September, just prior to autumn leaf abscission. At this time, only leaves from the previous autumn were selected. *Acer saccharum* leaves are easily degraded and may lose up to 73% of their weight in a single year while *F. grandifolia* leaves lose only about 20% (Michaelides, 1982). Only intact *A. saccharum* leaves were used, these being assumed to be not more than one year old. *Fagus grandifolia* leaves were classified by holding them up

to a low intensity light source. Leaves from the previous autumn (year-old leaves) were dark brown and partially translucent while older leaves were black, opaque, and did not transmit light (Fisher, 1979).

The second group (freshly-abscised leaves) was collected in early November, approximately three weeks after the end of autumn leaf fall. By this time all freshly-abscised leaves were completely submerged. These leaves were easily identified by their light brown coloration and the absence of deterioration. Fifty leaves were used for each experiment. Leaves were washed gently to remove mud or silt and to dislodge any attached aquatic animals. They were then placed in Petri dishes lined with moist filter paper and incubated as before to determine whether colonization had taken place. Each experiment was carried out twice.

RESULTS AND DISCUSSION

Colonization of leaves.—When leaves from the 5-min and 6-h exposures were examined, a thin surface film entrapping propagules and small pieces of debris was observed on the side previously exposed to the water. This film was not seen on leaves from the 24-h exposures, although germinating propagules were abundant on such leaves. At our test site, free-floating surface film readily adhered to the flat surface of a glass slide, trapping propagules beneath it. It is possible that the same mechanism initially holds propagules against the leaf prior to germination and subsequent colonization. The number of leaves on which *H. triglitzense*, *H. ontariensis* and *B. pulmonaria* propagules were found all increased from

TABLE I
CONTINUED

<i>Helicodendron triglitzense</i>	<i>Hormiactis ontariensis</i>		<i>Beverwykella pulmonaria</i>	
Sporulating	Present	Sporulating	Present	Sporulating
13.30 ± 3.33	18.00 ± 1.96	18.00 ± 1.96	1.00 ± 0	1.00 ± 0
30.50 ± 0.98	37.50 ± 4.90	30.50 ± 2.94	1.00 ± 0	1.00 ± 0
47.50 ± 0.98	73.10 ± 5.68	58.00 ± 1.98	5.50 ± 0.98	2.33 ± 0.92

the 5-min to the 24-h exposure (TABLE I). The number of leaves bearing *P. matsushimae* propagules was found to be approximately 82% after a 5-min exposure and this value did not vary significantly when exposure times were extended to 24-h.

After 14 days incubation, the number of leaves successfully colonized by these fungi was similar to the number with propagules initially present. Percentages of leaves colonized by *H. ontariensis*, *P. matsushimae* and *H. triglitzense* were positively correlated with exposure times. The number of leaves colonized by *P. matsushimae* increased slightly between the 5-min and the 6-h exposures but did not increase significantly after a further 18-h exposure. Sporulation occurred over the entire leaf surface and did not appear to be confined to any one portion of the lamina. No aero-aquatic fungi sporulated on control leaves (TABLE I). Our experiments indicate that the time elapsed between initial film-mediated propagule attachment, colonization and subsequent sporulation may be as little as one week (TABLE II). No fungi other than aero-aquatic species sporulated on the leaves.

The longer a leaf remains in contact with the water surface before sinking, the greater the changes that colonization by floating propagules will occur. Grazing aquatic organisms, e.g., snails, were frequently found on submerged leaves and may, by feeding on the propagules, have decreased the number of leaves successfully colonized.

Substrate preferences of aero-aquatic fungi.—*Pseudoaegerita matsushimae* and *H. ontariensis* were the two species most frequently found on freshly-abscised and year-old *A. saccharum* leaves but were generally more abundant on freshly-abscised leaves. *Helicodendron triglitzense* and *B. pulmonaria* were more frequent on year-old *A. saccharum* leaves than on freshly-abscised *A.*

saccharum leaves (FIG. 1). *Pseudoaegerita matsushimae*, *H. ontariensis* and *H. triglitzense* all sporulated more frequently on freshly-abscised *F. grandifolia* leaves than on year-old leaves, while *B. pulmonaria* exhibited no age preference (FIG. 2.).

Hormiactis ontariensis sporulated more frequently on freshly-abscised *Acer saccharum* and *F. grandifolia* leaves than on year-old leaves. *Pseudoaegerita matsushimae* was more frequent on freshly-abscised and year-old *F. grandifolia* leaves than on freshly-abscised and year-old *A. saccharum* leaves. *Beverwykella pulmonaria* sporulated more frequently on year-old *A. saccharum* leaves than on freshly-abscised leaves of this species but appeared to sporulate with equal frequency on freshly-abscised and year-old *F. grandifolia* leaves. *Helicodendron triglitzense* was found more often on year-old *A. saccharum* than on year-old *F. grandifolia* leaves but appeared more often on freshly-abscised *F. grandifolia* leaves than on freshly-abscised *A. saccharum* leaves (FIGS. 1, 2). In older *F. grandifolia* and *A. saccharum* leaves, sporulation occurred in dis-

TABLE II

TIME ELAPSED BETWEEN INITIAL INOCULATION OF *ACER SACCHARUM* LEAVES BY FLOATING PROPAGULES AND SUBSEQUENT DEVELOPMENT OF NEW PROPAGULES ON LEAF^a

Exposure times	% leaves with propagules from water surface	% leaves supporting sporulation after 1 week	% leaves supporting sporulation after 2 weeks
0	0	0	0
5 min	93 ± 3.92	5.0 ± 1.96	81 ± 7.84
6 h	90 ± 0	9.5 ± 2.94	81 ± 5.88
24 h	90 ± 3.92	9.5 ± 2.94	81.5 ± 2.94

^a Means of 3 replicates of 100 leaves ± 95% confidence interval.

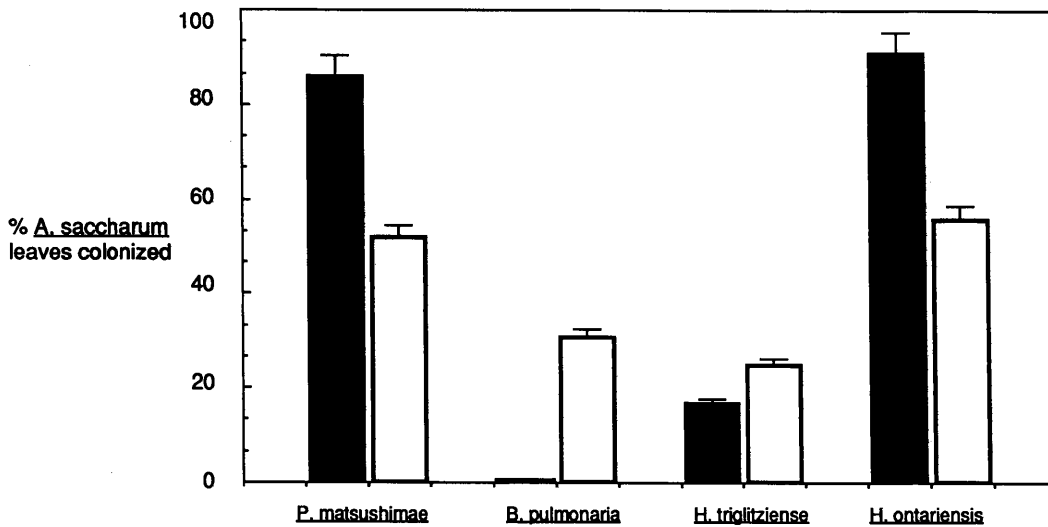


FIG. 1. Aero-aquatic fungi colonizing freshly-fallen and year-old *Acer saccharum* leaves. Means of two experiments each using 50 leaves, 95% confidence limits. ■, freshly-fallen leaves submerged approximately 3–4 weeks; □, year-old leaves submerged approximately 1 year.

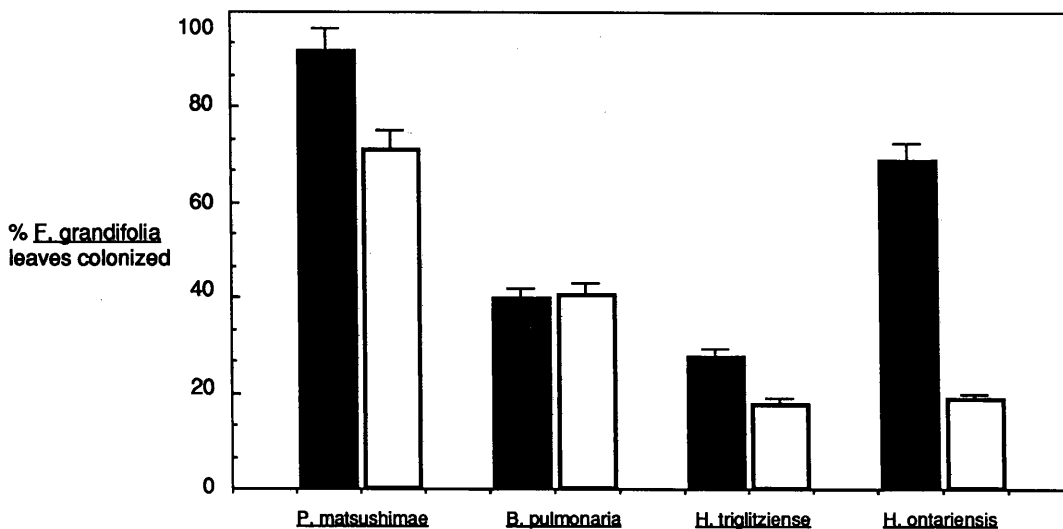


FIG. 2. Aero-aquatic fungi colonizing freshly-fallen and year-old *Fagus grandifolia* leaves. Means of two experiments each using 50 leaves, 95% confidence limits. ■, freshly-fallen leaves submerged approximately 3–4 weeks; □, year-old leaves submerged approximately 1 year.

crete but propagule-dense clumps approximately 4–6 mm in diam and were concentrated near the leaf spine. In freshly-abscised leaves sporulation was spread over the entire leaf surface.

The sporulation profiles (FIGS. 1, 2) of aero-aquatic fungi on freshly-abscised and year-old *A. saccharum* and *F. grandifolia* leaves are rela-

tively similar. Yet our data suggest that some aero-aquatic fungi sporulate more frequently on leaves of a particular age or species. Fisher (1979) found several species of aero-aquatic fungi that sporulated better on freshly-abscised *F. grandifolia* leaves than on year-old specimens and at least two other species that sporulated equally

well on freshly-abscised and decaying leaves. These data suggest that, as in many other substrates, a successional process may occur in leaves submerged in woodland ponds. Other factors, such as water temperature and pH, almost certainly influence this process but remain to be investigated.

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