The occurrence of myxomycetes in wood

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Abstract: Although the fruiting bodies of myxomycetes are commonly found associated with coarse woody debris in forest ecosystems throughout the world, there is no direct evidence that these organisms actually live within wood. In the research reported herein, surface sterilisation of pieces of wood taken from the branches of eight different tree species and the subsequent culturing of slivers of wood collected from within the interior of those branches clearly revealed the presence of the amoeboflagellates of myxomycetes. No evidence of the occurrence of plasmodia within wood was found, but amoeboflagellates emerged from 47% of the wood slivers placed in culture.

Keywords: amoeboflagellates, aphanoplasmodia, ecology, tree branches, wood density

Introduction

Myxomycetes are an important component of the assemblage of protozoans found in soil (Feest 1988, Stephenson and Feest 2014), and the methods to enumerate the populations of myxomycetes in soil and follow their population dynamics in response to environmental change were described by Feest and Madelin (1985a) and Feest (1988). Studies have shown that myxomycetes exist in soil as uninucleate amoeboflagellates that occur in one of three forms (amoebae, flagellates and cysts) and form plasmodia only when they are about to form fruiting bodies. These organisms appear to be important regulators of soil bacterial populations but this aspect of their ecology remains understudied.

The occurrence of myxomycetes within wood as part of the decomposition cycle has been generally assumed, but the actual recovery of myxomycetes from within wood, as opposed to the surface, appears to be limited to the report by Ostrofsky and Shigo (1981), who recovered *Comatricha aequalis* from discoloured living red maple (*Acer rubrum* L.) wood. They recovered a flagellate from the wood and cultured this until a fruiting body was

obtained. However, they were unable to repeat this. Myxomycetes are frequently observed fruiting on the cut surface at the ends of logs, and it certainly seems likely that they have emerged from within the log. Ostrofsky and Shigo (1981) concluded that myxomycetes were probably more prevalent in discoloured and decayed wood than was then known, but their role in this substrate was unclear. This can only be an assumption, since plasmodia are mobile and could have migrated from other situations. Plasmodia are frequently recovered in moist chamber cultures prepared with bark and wood, but this does not prove that they are living within the wood.

The objective of the research described herein was to show that not only are myxomycetes present within wood but they can be demonstrated to occur in the wood of more than one species of tree and it is possible show the form in which they exist in this substrate.

Materials and Methods

Fallen and decayed branches from a range of deciduous trees Sycamore (*Acer pseudoplatanus* L.), Spindle (*Euonymus europeaus* L.), Holly (*Ilex aquifolium* L.), Hazel (*Corylus avellana* L.) and Scots pine (*Pinus sylvestris* L.) and English yew (*Taxus baccata* L.) were collected from the forest floor at two localities near Bristol in the Southwest of the United Kingdom. These were collected over a period of the year that extended from January to June. Branches that were still attached and thus had not been in contact with the ground (aerial samples) also were collected. Each branch was identified to species on the basis of features of their bark, cross-sectional structure and attached leaves attached (if any were present). All branches were all of a similar size (ca 50 cm long and 4-6 cm diameter). The branches were taken directly to the laboratory, the bark removed and a section approximately 3-5 cm long collected in order to determine the water content and density of the wood. These lengths were weighed (fresh weight) and their volume calculated from measurements of

length and diameter. For irregular blocks, the volume was calculated from the displacement by pre-soaked blocks of wood submerged in a 0.005% Teepol solution. Density of the wood was determined by weighing dried blocks (drying at 60°C to a constant weight) and dividing this weight by the volume. This also allowed their water content to be calculated.

The remaining portions of the branches were surface sterilized by placing them in a 0.5% potassium permanganate solution overnight. As such, only wood unstained by potassium permanganate within the branches could be selected for sampling. After the surface sterilization process had been completed, the branches were drained and split open using a flamed blade in a sterile environment. From within each branch nine samples measuring approximately 1 x 0.2 x 0.2 cm were taken from the freshly cleaved surfaces and placed in 9 cm Petri dishes containing half-strength corn meal agar overlain with a dilute suspension of bacteria (*Aerobacter* sp.) and 2 cm^3 of sterile water. Three samples were placed on each plate and thus there were three plates per branch. The plates were placed in plastic bags and kept at 20°C. The surface fluid was observed weekly using phase contrast microscopy and x200 magnification. The 2 cm^3 of sterile water allowed the observation of swimming forms of myxomycete amoeboflagellates, which are highly distinctive. Later, plasmodia were observed, thus confirming that that these were indeed myxomycetes. Some of these plasmodia developed into fruiting bodies.

Results

Table 1 provides the full results of testing the branches for the presence of myxomycetes within wood. Forty-seven percent (34 of 72) the branches tested yielded amoeboflagellates. The density and moisture content of branches containing myxomycetes extended over the full range covered and thus was not a guide to the presence or absence of these organisms. For the type of tree (oak [*Quercus* spp.]) represented by the most samples, 18 of 29 samples (62%) yielded myxomycetes. Three of 12 aerial samples (25%) collected

from sycamore, ash and oak produced myxomycetes as did 31 of 60 ground samples (52%). In total, myxomycetes were recorded from eight different tree species. No plasmodia were observed that had not been preceded by amoeboflagellates, and of the 34 positive samples, 16 went on to form plasmodia. All of the latter were of the aphanoplasmodium type.

Discussion

The results presented herein clearly indicate that myxomycetes are present within wood, and in this substrate they take the form of uninucleate cells rather than the frequently assumed plasmodia. Clearly, the biological activity of myxomycetes in wood is based around the uninucleate form, and it would seem that the plasmodium is formed only when the appropriate conditions stimulate their formation prior to fruiting, although aphanoplasmodia seem well adapted to spread through wood. Of particular interest is that presence of myxamoebae within the wood of 25% of aerial samples (and in none of these were any other protozoans present although protozons were present in 73% (16 of the 22) of the positive ground samples), thus indicating that the formation of dry spores released into the air does indeed facilitate the colonisation of wood while the latter is still attached to the tree. Even the densest and least decayed wood contained amoeboflagellates, indicating further the early colonisation of wood. This has clear competitive advantages for myxomycetes and would suggest the reasons for the extensive formation of complex fruiting bodies.

Soil myxomycetes display a capability for the rapid formation of cysts, and these cysts can be stimulated into activity by freezing the soils (Feest & Stephenson 2015). Since none of these wood samples examined in the present study were tested after freezing, it remains to be determined if a similar situation involving a dynamic transition of cyst/non-cyst states exists in wood. If such a situation does exist, many positive samples may have been missed. In addition, the size of the sample tested (limited to nine 1 x 0.2 x 0.2 cm slivers of

wood) is small and it is possible that larger samples would have increased the rate of recovery.

Our results show that not only are myxomycetes present within wood of trees but this includes both wood that is still attached as well as wood that which has fallen to the forest floor. Moreover, the trees known to be colonized by myxomycetes include a wide range of different species and the probable form in which myxomycetes are present is the uninucleate form (amoebae, flagellates or cysts).

References

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Table 1. Summary data on wood examined for the presence of myxomycetes. Note: nd = no data.

Tree species	Collection number	Month collected	Aerial or ground	Myxomycetes present	Water content (%)	Density g/cm^3
Yew	1	January	G	No	10.86	0.60
Sycamore	2	January	G	No	17.34	0.41
Spindle	3	January	G	Yes	22.80	0.55
Spindle	4	January	G	Yes	42.49	0.61
Pine	5	January	G	Yes	46.88	0.53
Pine	6	January	G	Yes	42.07	0.78
Oak	7	January	G	Yes	30.95	0.60
Oak	8	January	G	Yes	19.85	0.48
Holly	9	January	G	No	18.03	0.33
Hazel	10	January	G	Yes	45.09	0.27
Hawthorn	11	January	G	No	21.49	0.60
Birch	12	January	G	Yes	nd	nd
Beech	13	January	G	No	63.81	0.21
Beech	14	January	G	No	40.91	0.44
Ash	15	January	G	Yes	52.23	0.39
Sycamore	16	March	A	No	nd	nd
Oak	17	March	G	No	nd	nd
Oak	18	March	G	Yes	nd	nd
Oak	19	March	G	No	nd	nd
Cherry	20	March	G	No	nd	nd
Beech	21	March	G	No	nd	nd
Beech	22	March	G	No	nd	nd
Beech	23	March	G	No	nd	nd
Ash	24	March	G	No	nd	nd
Ash	25	March	A	No	nd	nd
Sycamore	26	April	A	Yes	63.02	0.28
Birch	27	April	G	No	47.62	0.59
Birch	28	April	G	Yes	60.70	0.35
Oak	29	April	G	Yes	69.94	0.28
Oak	30	April	G	Yes	72.17	0.25
Oak	31	April	G	No	79.73	0.22
Oak	32	April	G	No	71.54	0.23
Oak	33	April	G	Yes	61.36	0.43
Birch	34	April	G	Yes	74.14	0.30
Birch	35	April	G	No	35.99	0.56
Ash	36	April	A	No	31.82	0.36
Ash	37	April	G	Yes	36.13	0.41
Ash	38	April	A	No	19.35	0.43
Ash	39	April	G	No	34.07	0.41
Ash	40	April	A	No	57.63	0.25

Birch	41	May	A	No	86.99	0.10
Birch	42	May	G	No	71.77	0.30
Pine	43	May	G	No	27.27	0.42
Oak	44	May	G	No	52.35	0.42
Oak	45	May	G	Yes	67.77	0.27
Oak	46	May	G	Yes	40.38	0.30
Oak	47	May	G	No	63.72	0.37
Oak	48	May	G	Yes	82.47	0.19
Birch	49	May	G	No	66.56	0.34
Ash	50	May	G	Yes	62.16	0.44
Ash	51	May	G	No	73.19	0.31
Oak	52	June	G	No	31.20	0.53
Oak	53	June	G	Yes	74.63	0.30
Oak	54	June	G	Yes	79.46	0.18
Oak	55	June	G	No	37.72	0.37
Ash	56	June	G	Yes	61.75	0.32
Ash	57	June	A	No	16.89	0.62
Ash	58	June	A	Yes	15.63	0.63
Oak	59	June	G	No	11.02	0.19
Oak	60	June	G	Yes	12.00	0.18
Oak	61	June	G	Yes	56.73	0.34
Oak	62	June	G	No	76.89	0.20
Oak	63	June	G	Yes	27.87	0.38
Oak	64	June	G	Yes	41.54	0.40
Oak	65	June	G	Yes	47.57	0.46
Oak	66	June	G	No	37.04	0.60
Oak	67	June	G	Yes	57.30	0.48
Oak	68	June	G	Yes	30.67	0.44
Oak	69	June	G	Yes	26.09	0.30
Oak	70	June	A	No	52.83	0.43
Oak	71	June	A	No	15.19	0.59
Oak	72	June	A	Yes	29.33	0.54