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Repeated damage results in polarised development of foraging mycelial systems of *Phanerochaete velutina*

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Abstract

Mycelial cord systems of *Phanerochaete velutina* growing from wood inocula across non-sterile soil were subjected to disturbance by repeated (6–9-day intervals) complete severance and removal of cords emerging from three, four or zero (control) vertical sides of the inoculum. Regrowth occurred after removal from four sides, though morphology, quantified by fractal dimension (D_{BM}), did not differ from controls. After removal from three sides, mycelial regrowth occurred and the D_{BM} values of both intact and regrown parts of the mycelium were initially the same. Following subsequent removal, limited regrowth occurred, i.e. systems became polarised towards the intact mycelium. The proportion of regrowth from three disturbed sides altered, mycelial area fell from 72% to 30–50% of regrowth from inocula disturbed at all four sides. The reduced regrowth also had lower D_{BM}s than the intact mycelium developing from the undisturbed side of the inoculum. Mycelial extension rate of all regrowth from severed sides was slower than undisturbed mycelium. Decay of inocula was greater, though not significantly, where extra-resource mycelium was most extensive.

Keywords: Mycelial foraging; Cord; Fractal; Image analysis

1. Introduction

Mycelial cord systems of woodland saprotrophic basidiomycetes, which are found interconnecting dead organic resources at the soil/litter interface, are often extensive (many m^2) and long-lived (many years) [1,2]. It is likely that persistent and extensive mycelial systems will be periodically exposed to either enrichment or destructive disturbance [3]. Enrichment disturbance has been examined in detail for a number of saprotrophic cord-forming fungi, as either localised addition of uncolonised resources [4–7] or widespread addition of nutrients to soil [8–10].

To date only a few studies have examined the effect of destructive disturbance on mycelial systems, and these have been largely qualitative [11,12]. Effect of removal of colonised wood resources on mycelial networks has been examined in laboratory soil systems [11] and disruption of mature annuli of the fairy ring fungus *Clitocybe nebularis* examined in the field [12]. Severing of mycelial cord systems from host plants led to rapid decline of respiratory activity in ectomycorrhizal fungi in microcosms [13]. Also effects of population explosions of Collembola

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on mycelia have been noted in laboratory microcosms – intensive grazing can completely destroy cord systems, while less intensive grazing can considerably influence morphology ([14], A. Morgan and L. Boddy, unpublished).

Currently the extent to which mycelial loss influences system development is poorly understood, but it may play a significant role in determining mycelial foraging patterns. In this paper we report on the results of a laboratory study which investigated mycelial responses of *Phanerochaete velutina* to repeated episodes of destructive disturbance at specific locations in cord systems emanating from wood blocks in non-sterile soil.

2. Materials and methods

2.1. Fungal isolates

Laboratory stocks of *P. velutina* were used, originally isolated from decaying beech wood from the Forest of Dean, Gloucester, UK (National Grid ref. SO611145). The isolate was maintained and routinely subcultured on 2% (w/v) malt agar (MA; 20 g Munton and Fison spray malt, 15 g Lab M no. 2 agar l^{-1} distilled water).

2.2. Flask culture and inoculum preparation

Beech wood blocks (4 cm^3) cut from a freshly felled tree and frozen until required, were defrosted, soaked overnight in distilled water, autoclaved at 121°C for 30 min and reautoclaved after 24 h.

Inocula were prepared by adding 30 4-cm^3 beech blocks to 2-week-old cultures of *P. velutina* on 500 ml MA in 2-l wide-necked conical flasks. After 30 days at 20°C in darkness colonised inocula were scraped free of mycelium and agar and placed in the centre of soil trays.

2.3. Soil trays

Topsoil (10–15 cm), cleared of wood and leaf litter, was collected from a beech woodland in Coed Beddick Inclosure (National Grid ref. SO528018), sieved to <4 mm and air-dried at room temperature. Soil matric potential was adjusted to -0.02 MPa [15] with distilled water, and wetted soil (200 g) was compacted in square, lidded plastic bioassay trays (24×24 cm, Nunc: Gibco, Paisley, UK).

2.4. Experimental design

Experiments were performed to determine the effect of repeated disturbance on exploratory cord systems by cutting regions of extending mycelia. Mycelium was removed by severing mycelial-inoculum interconnections with a scalpel and lifting the excised mycelium from the soil surface with fine forceps. Three treatments were performed: (i) all mycelium was removed; (ii) mycelium was removed from three inoculum sides and (iii) undisturbed mycelium as control. One treatment was applied to each mycelial system, with five replicates per treatment, immediately after soil tray photography at 6–9-day intervals for 42 days.

2.5. Photography and image capture

Soil trays were photographed from 60 cm height at 6–9-day intervals, immediately prior to treatment application, using an Olympus OM 10 camera body fitted with a 50-mm OM-system macro lens and Ilford FP4 (125 ASA) 35 mm black and white film. Films were developed and printed to 10×10 cm with maximum contrast between soil and mycelial cords. Images were captured onto a framestore (Synapse, Synoptics, Cambridge, UK) by a Hitachi KP-M1 monochrome CCD video camera fitted with a Canon TV macro-zoom lens, linked to an Optiplex GXMT 5100 microcomputer (Dell Systems, Wicklow, Republic of Ireland). Images were subsequently analysed using image processing software (SEMPER for Windows version 6, Synoptics, Cambridge, UK). Images were analysed for radial extent, mycelial cover and fractal dimensions of extra-resource mycelia [16].

2.6. Quantification of mycelial growth and distribution

Pixel lines were calibrated against known lengths and, for each image, radial extent was determined from radial measurements from the inoculum to

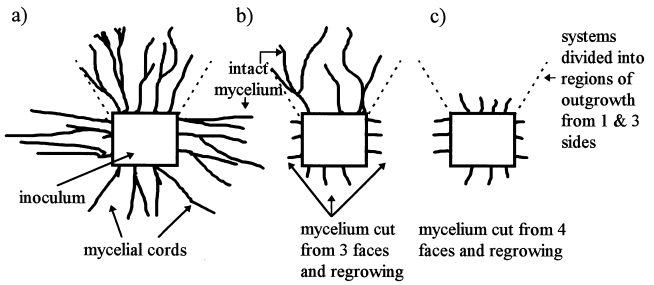

Fig. 1. Diagram of removal treatments applied to mycelia extending from inoculum blocks. a: Undisturbed system. b: Mycelium removed from three sides. c: Mycelium removed from all sides.

the mycelial margin. Mycelial cover (mm^2) was determined from white pixel areas of extra-resource mycelial cord systems [16,17].

Mycelial distribution was quantified by box-count fractal dimension [16] whereby a series of grids containing boxes of varying side length from 3 to 61 pixels were laid over each processed image of the mycelium and the number of boxes for each box size intersecting the mycelium counted. The box count fractal dimension was derived from the regression line gradient of a double logarithmic plot of the log number of intersected boxes ($N(s)$) against log of box side length (s) [16].

The 'separate particles' facility in the SEMPER program allowed mycelial regions to be separated from one another (Fig. 1). Thus the development from one inoculum side and three inoculum sides could be analysed separately for all treatments including controls. The mass fractal dimension (D_{BM}) for these separate regions was determined for mycelial systems at 16 and 35 days for all treatments.

2.7. Estimates of decay rate

Initial resource decay state was estimated as the relative density (RD, g cm^{-3}) of five colonised wood blocks. At the end of the experiment all resources were used to estimate final RD from which the rate of decay (g cm^{-3} d^{-1}) was determined.

2.8. Statistical analysis

A randomised block analysis of variance (ANOVA) was used to detect treatment effects. Means were compared using a Scheffé test.

3. Results

3.1. General observations of mycelial systems

Before any disturbance, all mycelia were morphologically similar, extending radially from inoculum blocks (Fig. 2a,e,i). After removal of mycelium

Table 1
Mean mass fractal dimension of mycelia extending from either one or three sides of inoculum blocks after either removal of all mycelia, removal of mycelia from three sides of inocula or undisturbed control, after 16 or 35 days

Time (days)	Mycelial treatment	D_{BM} of mycelia from one inoculum side (S.E.M.)	D_{BM} of mycelia from three inoculum sides (S.E.M.)
16	All removed	$1.594^{a,1}$ (0.015)	$1.665^{a,2}$ (0.015)
	Removal at three sides	$1.609^{a,1}$ (0.017)	$1.614^{a,1}$ (0.015)
	Undisturbed	$1.641^{a,1}$ (0.019)	$1.668^{a,1}$ (0.032)
35	All removed	$1.556^{a,1}$ (0.022)	$1.599^{a,1}$ (0.032)
	Removal at three sides	$1.563^{a,1}$ (0.036)	$1.391^{b,1}$ (0.074)
	Undisturbed	$1.562^{a,1}$ (0.065)	$1.622^{a,1}$ (0.032)

At each time, means in the same column followed by the same letter or in the same row followed by the same number are not significantly different ($P > 0.05$) using randomised block ANOVA and Scheffé tests.

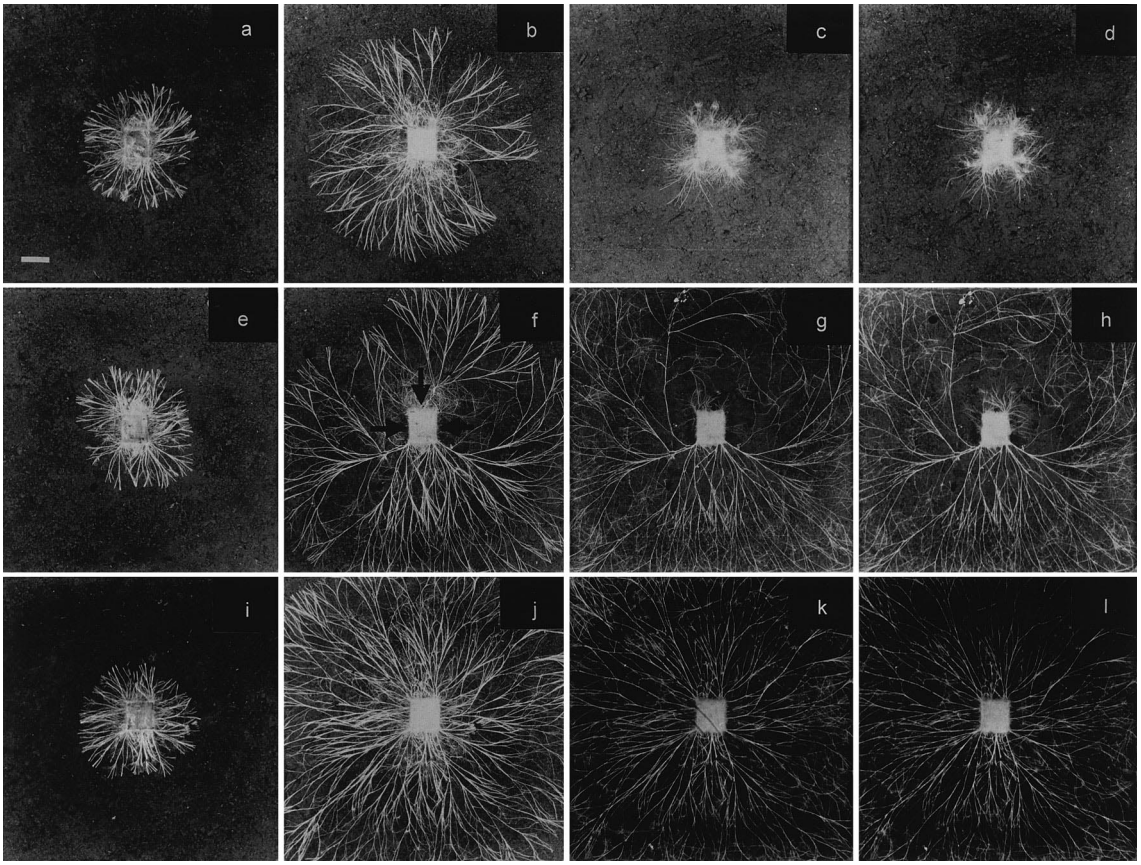

Fig. 2. Mycelial systems of *P. velutina* extending from 4-cm^3 beech inocula prior to first destructive disturbance at 7 days (a, e, i); and subsequent removal at 16 days (b, f, j); 30 days (c, g, k) and 35 days (d, h, i). Mycelium was either removed from inocula at all four sides (b–d), on three sides (arrowed) (f–h) or left intact (i–l). Scale bar is 2 cm. The effect of each treatment, applied immediately after trays were photographed, is shown in the following photograph, i.e. prior to the next treatment application.

from all four inoculum sides, a renewed flush of corded mycelium extended from each inoculum beyond a more diffuse central mycelium (Fig. 2b). Repeated regrowth occurred after each removal of mycelium, typically predominantly from inoculum block corners (Fig. 2c,d) although the regrowth was irregular. When mycelium was removed from three inoculum sides (Fig. 2f–h), corded regrowth occurred, although initially primarily from the side opposite the remaining undisturbed mycelial region (Fig. 2f). Lateral regrowth was limited and of diffuse morphology. Subsequent regrowth after repeated removal of mycelium was minimal and mycelial cords from the intact mycelial region rapidly extended into unoccupied territory (Fig. 2g,h). Control systems continued to extend radially until tray sides were reached, after which mycelial systems thinned (Fig. 2j–l).

3.2. Disturbance effects on mycelial morphology

Fractal dimension (D_{BM}) of mycelial cords extending from both one and three inoculum sides of undisturbed controls did not differ significantly ($P > 0.05$) after 16 or 35 days (Table 1). In systems which had mycelia removed at either three or four inoculum sides, D_{BM}s of mycelia regrown from one side were similar ($P > 0.05$) at 16 days to mycelia growing from single sides of undisturbed controls (Table 1).

In disturbance treatments where mycelium had been removed from all four inoculum sides the

D_{BM} of mycelial regrowth extending from one side was significantly lower ($P \leq 0.05$) at 16 days, compared to regrowth from three inoculum sides (Table 1).

Mycelia regrown from inocula with three disturbed sides had a significantly ($P \leq 0.05$) lower fractal dimension after 35 days, compared to mycelia growing from three sides of either controls or from inocula where all mycelia had been repeatedly removed and had regrown (Table 1).

3.3. Effect of mycelial removal on area cover, extent and resource decay

Total mycelial cover did not differ ($P > 0.05$) between systems before application of first removal treatment at 7 days, although mycelial cover from one inoculum side was significantly ($P > 0.05$) less than from four sides (Fig. 3). After disturbance, mycelial cover in undisturbed systems with was significantly ($P \leq 0.05$) greater than in systems with repeated removal of all mycelia (Fig. 3).

Mycelial cover of regrown mycelia was greatest after application of the first removal treatment at 7 days, and regrowth subsequently declined following further disturbance treatments (Fig. 3). The area covered by mycelia regrown from three inoculum sides was significantly ($P \leq 0.05$) less than mycelial regrowth from all four sides (Fig. 3). However, the proportion of regrowth from three disturbed sides changed with time, mycelial area fell from an initial 72% of the cover from four inoculum sides (prior to disturbance) to only 30–50% of regrowth from inocula disturbed at all four sides (Fig. 3). This reduction in regrowth from three disturbed sides coincided with mycelial area of the remaining intact mycelium, growing from one face, reaching area coverage of undisturbed controls ($P > 0.05$, Fig. 3).

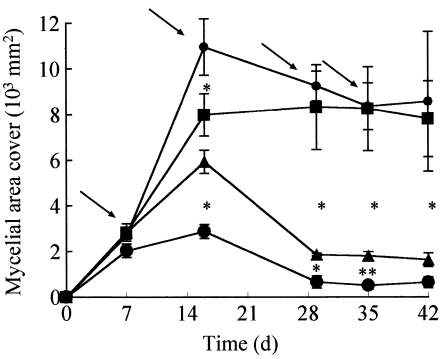

Fig. 3. Time course of development of mycelial area of *P. velutina* systems extending from 4-cm^3 beech wood inocula as affected by destructive disturbance: intact control (●); regrowth following removal of all mycelium (▲); intact mycelium (three other sides removed) (■); mycelial regrowth following removal from three faces (●). Error bars show the standard error of the mean of five replicates; significant differences between treatments are indicated by asterisks; $*P \leq 0.05$; $**P \leq 0.01$. Arrows indicate time of treatment application.

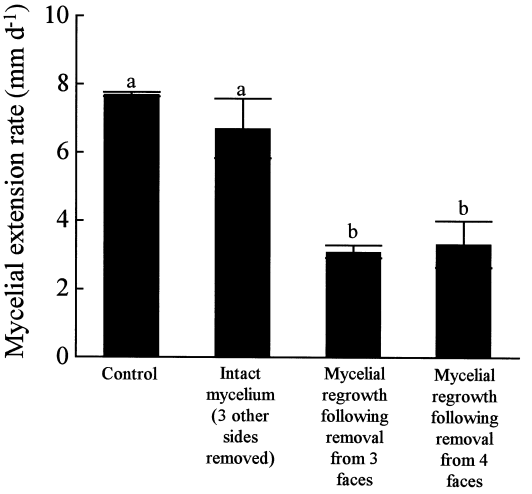

Fig. 4. Extension rate of *P. velutina* mycelia developing from 4-cm^3 beech wood inocula following destructive disturbance. Error bars show the standard error of the mean of five replicates; significant differences ($P \leq 0.001$) between treatments are indicated by different letters.

Extension rates (to 16 days) of mycelial regrowth from either three or four severed sides was only half of the rate of the mycelium extending from controls and intact mycelia in systems where severing occurred ($P \leq 0.001$, Fig. 4).

Inoculum decay rates (2.4–4.2 mg cm^{-3} d^{-1}) did not differ significantly ($P > 0.05$), although the trend was for more decayed inocula which supported greater intact mycelium.

4. Discussion

This is the first paper to demonstrate quantitatively polarised mycelial development in saprotrophic cord-forming fungi as a result of destructive

disturbance. Polarised growth as a result of enrichment disturbance, i.e. input of partly colonised or uncolonised material [3] has been demonstrated for *Hypholoma fasciculare*, *Steccherinum fimbriatum* and *P. velutina* [4–6]. With the latter, polarity was only manifest when the added resources were sufficiently larger or of higher quality than the supporting resource [18]. However, recently a study performed on nutrient depleted soil showed polarity of mycelial 'patch' formation by *P. velutina* when wood baits of equal size to the inoculum were added behind the growing front [17].

P. velutina clearly had the ability to regrow mycelium from inocula following destructive disturbance, although continued disturbance altered rate and the pattern of regrowth, as shown by reduced extension and fractal dimension. The general reduction in mycelial extension from disrupted sites may be due to physical damage, which at the hyphal level is rarely catastrophic but delays hyphal extension [19]. Damaged areas may also be rapidly sealed off by plugging of septal pores [20].

Decomposer mycelial systems have been shown to alter soil conditions in the field [21] and in soil microcosms (S. Owen and L. Boddy, unpublished), and although possible, it is unlikely that repeated growth over soil areas would create soil conditions suppressing mycelial development. On the contrary, mycelial regrowth over previously explored territory has been observed from both intact mycelial cords [17] and from inocula (D.P. Donnelly, L. Boddy and J.M. Wells, unpublished). Re-exploration of an area increases the probability of encountering and therefore exploiting fresh litter inputs to a region [17].

The reduced mycelial regrowth may indicate a switch between functional modes in different parts of the same mycelium [22]. Unstressed regions maintain a rapidly extending exploratory mode, whilst the disrupted system has slow growth, and may represent a mode tolerant to environmental stress [23]. Differences in growth form and extension rate within mycelial colonies have been seen previously in many fungi, usually on artificial media and under diverse conditions [23].

Mycelial regrowth has also been shown in this study to be affected by the development of intact mycelial regions. After the first severance, total regrowth from three inoculum sides was, as might be expected, approximately 75% that regrown from four sides initially. However, as the intact extra-resource mycelium continued to develop, the amount of mycelial regrowth after subsequent cuttings was significantly reduced compared to when there was no intact mycelium present. The regrown mycelium was also more irregular morphologically compared to mycelium regrown from all four inoculum sides. The intact mycelium was evidently able to continue growth at the expense of the disturbed sites, leading to the reduction in amount and fractal dimension of regrowth.

Asymmetrical foraging systems have previously been shown to occur in *H. fasciculare* as a result of destructive disturbance of cord systems [11]. Severance of inoculum-bait interconnections and selective removal of resources led to shifts of exploration to undisturbed regions, eventually leading to colonisation of all remaining resources [11]. However in the latter study, beech wood blocks were the foci for mycelial development, whereas in this study, changes to the growth of disturbed mycelial regions was due to the continued development of an intact mycelial region.

The driving force of mycelial development in systems of both cord-forming fungi and woodland fairy-ring fungi may be the sink strength of the foraging front [12]. There are however, major differences in the morphology of these two types of saprotrophic foraging mycelial system especially as woodland fairy-ring fungi only produce limited cords, which for *C. nebularis*, at least, are unable to traverse bare soil [12]. In undisturbed cord systems, the radially expanding system will probably have balanced sinks at the expanding margin. However, symmetry may be broken by either encounter with available resources [4–6] or due to mycelial destruction as demonstrated here and for *H. fasciculare* [11]. Repeated removal of mycelium from three inoculum sides allowed the mycelium growing from the undisturbed side to develop more extensively, possibly forming the 'dominant-sink' part of the mycelial system. It is recognised that mycelial size may determine the success of an individual during competition between species [24], and in this study the large intact extra-resource mycelium may regulate further mycelial outgrowth in other regions of the same individual. The balance of competition for resources

between foraging fronts linked through the inoculum may be altered by destructive disturbance. Control of growth in unsuccessful areas may be similar to regression of non-connected cords whilst resource-connected cords continue to develop. Also within annuli of fairy rings, inner trailing edge mycelia undergoes autolysis as the outer edge mycelia continues to extend [4–6,12].

This study has further demonstrated both the degree of interconnectedness and autonomy within mycelial networks, which has previously been seen in systems subjected to enrichment disturbance. Previous studies [1,25,26] have indicated the importance of spatial distribution of resources in determining pattern of mycelial networks. It is likely that the complexity of mycelial networks found within the woodland ecosystem, will also be largely influenced by destructive disturbances.

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