Ionizing radiation attracts soil fungi

Nelli N. ZHDANOVA[1], **Tatyana TUGAY**[1], **John DIGHTON**[2]*, **Victor ZHELTONOZHSKY**[3] **and Patrick MCDERMOTT**[4]

[1] *Institute of Microbiology & Virology, National Academy of Sciences of the Ukraine, Kiev 25214, Ukraine.*
[2] *Rutgers University Pinelands Field Station, New Lisbon, New Jersey 08064, USA.*
[3] *Institute for Nuclear Research, National Academy of Sciences of the Ukraine, Kiev 03028, Ukraine.*
[4] *Rutgers Environmental Health and Safety, Rutgers University, Piscataway, New Jersey 08854, USA.*
E-mail : dighton@camden.rutgers.edu

During the last 15 years, about 2000 strains of 200 species of 98 genera of fungi have been isolated from around the Chernobyl Atomic Energy Station. Many of these microfungi are capable of growing into and decomposing 'hot particles'; carbon based radioactive graphite from the reactor and there are suggestions that some fungi actively direct their growth toward sources of radioactivity, possibly attracted to the carbon skeleton of these structures. In our experiments, we eliminated the confounding effects of carbon as a fungal resource, by developing experimental protocols that expose fungal spores and their germinating hyphae to directional sources of ionizing radiation allowing us to measure fungal response to ionizing radiation *per se*. We show that both beta and gamma radiation promote directional growth of hyphae towards the source of ionizing radiation.

INTRODUCTION

Recent work from in and around the remains of the Chernobyl Atomic Energy Station (ChAES) demonstrates that soil micro fungal communities have been altered by the intense radiation fallout, leading to simpler community structure and a dominance of melanin-containing (pigmented) fungal species (Zhdanova *et al.* 1995). During the last 15 years about 2000 strains representing 200 species of 98 genera of fungi have been isolated from around the ChAES (Zhdanova *et al.* 2000). Depending on location, these isolates have been subjected to a wide range of ionizing radiation doses, up to 700 Gy h^{-1} ($70\,000 \text{ mR h}^{-1}$). Some of these fungal species have incurred altered genetic composition as a result of exposure (Mironenko *et al.* 2000).

Some of these microfungi are capable of growing into and decomposing 'hot particles'; carbon based radioactive graphite from the reactor (Zhdanova *et al.* 1991). Observations of the growth of fungal hyphae towards hot particles has led to the suggestion that the fungi may perceive and respond to the presence of ionizing radiation *per se* and, thus, are not only attracted to the carbon skeleton of these structures (Vember *et al.* 1999). To date these observations have been subjective and at best determined from counts of numbers of emergent hyphae growing in the direction of the source of ionizing radiation in comparison with number growing away from the source. This paper provides evidence from measures of the angle of axis of hyphal growth relative to the line of trajectory of the main beam of ionizing radiation. Statistical analysis of comparative mean angles of axis of growth provide confidence in the favored directionality of growth. A demonstration of the ability of microfungi to detect and respond to the presence of ionizing radiation, resulting in directed growth or positive radiotropism, would show a novel ecological response of fungi, indicating their ability to sense and respond to ionizing radiation. Given the previous interest in the role of fungi in radionuclide accumulation and turnover in the environment (Haselwandter 1978, Dighton & Horrill 1988, Murumatsu *et al.* 1991, Dighton & Terry 1994, Haselwandter & Berreck 1994), the mechanisms whereby fungi arrive at sources of radionuclides in the environment and the processes that occur during the decomposition/modification of these sources are ecologically important in regulating radionuclide movement and for potential site remediation.

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* Corresponding author.
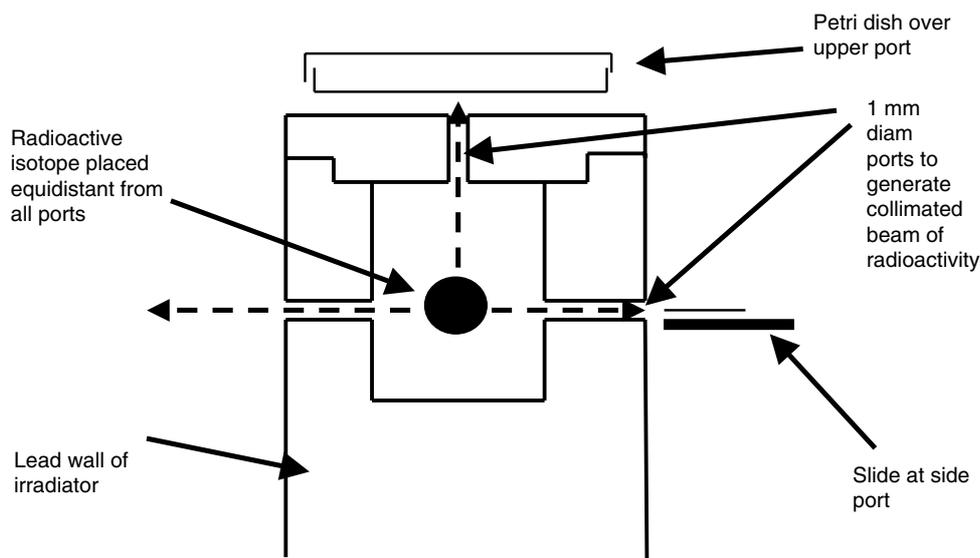
Fig. 1. Diagrammatic representation of the irradiators used in this study (not to scale). The walls of the irradiator are constructed from a solid block of lead. The lid is recessed to avoid the possibility of emissions 'leaking' along the lines of the join with the body of the irradiator. The central well was designed to position the radioactive source material equidistant from the top and each of the four side ports. The diagram is shown with a Petri plate over the top port and a microscope slide and coverslip adjacent to a side port to indicate the positions in which cultures were exposed to the ionizing radiation.

To evaluate the role of ionizing radiation on the directional growth of microfungi, we eliminated the confounding effects of carbon as a fungal resource by developing experimental protocols that expose fungal spores and their germinating hyphae to directional sources of ionizing radiation allowing us to measure fungal response to ionizing radiation *per se*. We show that, in some species, radiation promotes directional growth of hyphae towards the source of ionizing radiation.

METHODS

Fungal isolation and maintenance

Mitosporic fungi were isolated from a variety of locations in the Ukraine. Some of these locations were from radioactively contaminated regions (the 4th Block of the ChAES, hot particles in soil near the ChAES and from soil of the Red Forest, which is adjacent to the ChAES). Other species were isolated from less contaminated areas, either before the explosion or from remote sites, including the New Jersey pine barrens. Fungal cultures were maintained on Chapek Dox agar, until used in experimental procedures. Representate strains are maintained in the culture collection of the Institute of Microbiology and Virology, Kiev.

Irradiation

Radioactive emissions from selected sources were collimated using a cuboid lead irradiation box consisting of a central well housing the radioactive source (^{32}P for beta emissions, ^{109}Cd for gamma emissions, both at a source activity of approximately 2×10^8 Bq), and 1 mm diam holes drilled through the 2 cm lead walls in each of the four sides and the lid (see Fig. 1). Fungal cultures were presented to these collimated beams of radioactivity as conida. The conidia were either (1) suspended in sterile deionized water in a cavity slide and placed over the top port, or (2) on water or Chapek Dox agar (with reduced, 100 mg l^{-1}, or no sucrose) in a 60 mm diam Petri dish and taped to the top or side ports, or (3) grown as mycelial culture on slabs of Chapek Dox agar cut from Petri dishes and placed on microscope slides and covered with a coverslip. In this way, the behaviour of spore germination, emergent hyphal growth, and subsequent hyphal growth could be measured in response to directional sources of radiation. Irradiation times were for 24–48 h at laboratory temperature (approx. 20 °C). These times reflect the difference in time to germinate between the spores of the different fungi. Most of the investigated species in this study (Table 1) had the same growth and germination rate (20–24 h). However, *Cladosprium sphaerospermum* grew at a slower rate that than the rest, requiring 48 h incubation. Here we report solely the directional growth of hyphae to gamma and beta radiation.

Image analysis

The impingement point of the collimated beam of radioactive emissions was marked on the microscope slide or Petri dish. Digital photomicrographs (Nikon CoolPix 900) were taken before and after irradiation at the point of impingement and at defined distances away from that point. Images were analyzed using

Table 1. Synopsis of fungal isolates used, their source of isolation and visual observations of directional growth to radioactivity in previous studies.

Species	Isolate	Place and time of isolation	Radioactivity of substrate at isolation time	Tropism discovered under exposure radioactivity (Bq)
Penicillium hirsutum	3	Soil hot particles May 2002	1.5×10^6 Bq	3.5×10^4 6.5×10^4
P. hirsutum	1	Soil hot particles May 2002	6.0×10^4 Bq	3.5×10^4
P. lanosum	10	The 4th block ChNPP May 2002	20 mR h^{-1}	3.5×10^4
P. westlingii	8	The 4th block ChNPP May 2002	20 mR h^{-1}	1.5×10^4
Cladosporium cladosporioides	10	The 4th block ChNPP May 2002	20 mR h^{-1}	3.5×10^4 6.5×10^4
C. cladosporioides	396	Black earth soil, 1957	Control	No tropism 3.5×10^4 6.5×10^4
C. cladosporioides	4	Soil of 10-km zone of ChNPP, 1986	4.05×10^7 (Bq kg^{-1})	8.0×10^4
C. cladosporioides	5	Reactor graphite particles, 1994	3.6×10^6 (Bq kg^{-1})	5.3×10^4 8.1×10^4
C. sphaerospermum	60	The 4th block ChNPP, 1997	0–50 Bq cm^{-2}	8.1×10^4
C. sphaerospermum	3176	Sod-podzol soil, 1997	Control	No tropism 3.5×10^4 6.5×10^4
Paecilomyces lilacinus	1941	Soil of 10-km zone of ChNPP, 1994	3.2×10^4 (Bq kg^{-1})	10.0×10^4
P. lilacinus	101	Crimea, soil, 2000	Control	No tropism 3.5×10^4 6.5×10^4
Penicillium roseopurpureum	147	Soil of 10-km zone of ChNPP, 1987	1.4×10^6 (Bq kg^{-1})	7.5×10^4 10.0×10^4
P. roseo-purpureum	100	Air, library, 1999	Control	No tropism 3.5×10^4 6.5×10^4

the software package Image-Pro Plus (Media Cybernetics®) to provide the 'return angle'. We define the return angle as the angle made between the point of impingement of radioactivity in the culture vessel and the direction of growth of the distal portion of the emergent hyphum from each spore (Fig. 2). A low return angle ($<90°$) indicates mean hyphal growth towards the source of radioactivity and a high angle ($90–180°$) away from the source.

Statistical analyses

Hyphal return angles were measured on multiple conidia (30–50) from replicate experiments (usually three) using the same fungal isolate. Comparisons were made between fungal reactions to radiation exposure and replicates incubated in the absence of radiation (controls) using the GLM routine of SAS to perform analysis of variance. Means separation were calculated using Tukey's Honestly Significant Difference Test at an alpha level of 0.05.

RESULTS

We investigated 27 responses of interactions between fungal isolates and radiation source. Of these, 18 (66.7%) showed positive stimulation of growth towards the radiation source (low mean return angle), and eight showed no response. Of these, 69% of the fungi exposed to ^{109}Cd and 64% to ^{32}P showed preferred growth towards the source of ionizing radiation. Of fungal species isolated from radioactively contaminated sites, 86% of the interactions showed positive directional growth to the radiation beam, whereas of the limited number of fungi isolated from less contaminated areas (e.g. *Cladosporium sphaerospermum* 3176, *Penicillium roseopurpureum* 100, *Paecilomyces lilacinus* 101) showed directed growth in only one case.

Due to the restricted number of irradiators that were available (two), it was not possible to conduct directly comparative experiments between exposure to β- and γ-radiation for each species at the same time. Hence Fig. 3 shows responses of fungi to γ- and Fig. 4 to β-radiation. Where exposure to both sources of radiation occurred simultaneously, the responses to both ionizing radiations presented on the same graph (Fig. 5).

Examples of results showing the mean 'return angle', representing the direction of hyphal growth toward the source of radiation are given in Fig. 3 for a comparison of response to ^{109}Cd (gamma). Here we see significant directed gowth to the source of radiation by *Penicillium roseopurpureum* 147 (from contaminated Red Forest soil), *P. hirsutum* 3 (rom hot particles), *Cladosporium cladosporioides* isolates 60 and 10 (from the 4th Block reactor room). *C. sphaerospermum* 3176, although isolated from control, uncontaminated soil, also showed a positive response. No significant directional growth
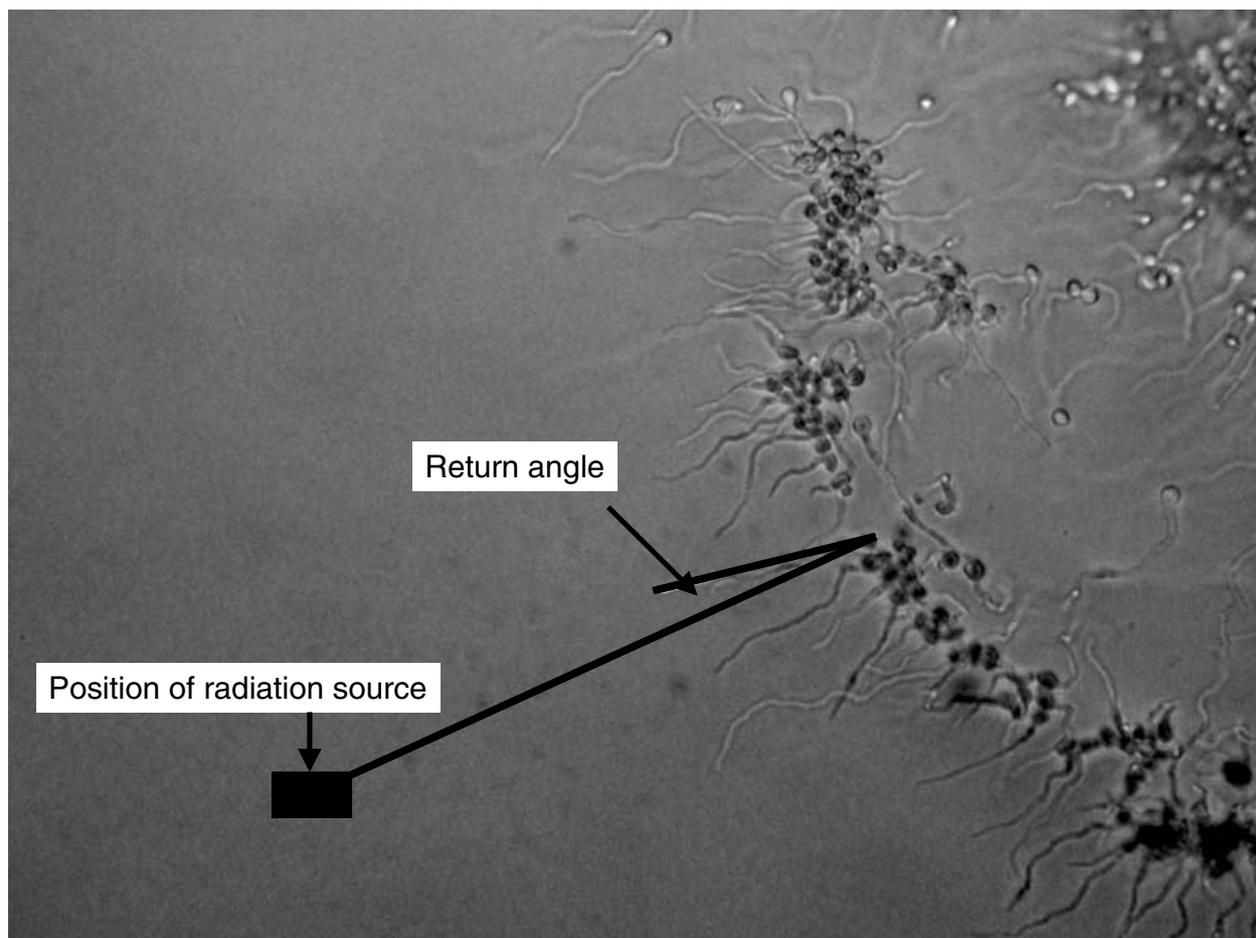
Fig. 2. Digital photomicrograph of germinating fungal spores indicating the way in which image analysis was performed to calculate the 'return angle'. The position of the source of ionizing radiation was placed according to X–Y coordinates. Using Image Pro Plus software a line from the source to the spore and a line along the axis of the most distal portion of the emerging hypha was drawn. The software automatically determines the angle between these two lines even if they do not actually intersect.

was seen in *C. cladosporioides* 396 and *Paecilomyces lilacinus* 101 which were both isolated from uncontaminated soils. However, no directional growth response was also seen in *Penicillium lanosum* (from the 4th Block) and *Paecilomyces. lilacinus* 1941 (Red Forest soil), both of which were originally isolated from areas of high levels of radiation.

Fig. 4 shows growth response of emerging hypahe to ^{32}P (beta) radiation. Significant directed growth was seen in *P. roseopurpureum* 147 (Red Forest soil), *P. lanosum* (4th Block), *P. hirsutum* 3 (hot particles) and *C. cladosporium* 60 (4th Block). No directional growth response was seen in *Paecilomyces lilacinus* 101, *C. cladosporium* 396, *Penicillium roseopurpureum* 100 or *C. sphaerospermum* 3176, which were all isolated from uncontaminated locations. *Paecilomyces lilacinus* 1941 and *Penicillium westlingi* were the only species isolated from a radioactively contaminated sites (Red Forest and 4th Block respectively) that did not show directional growth response to β-radiation.

Fig. 5 shows the growth response of emergent hyphae between radionuclide sources where both fungi

were exposed at the same time in a direct comparison. Here, *C. cladosporioides* 4 (isolated from the 10 km zone around Chernobyl before the accident) and all other isolates from radioactively contaminated areas all show directional growth towards both β- and γ-sources of radiation. The response by *Penicillium hirsutum* 1 is, however, not statistically significant. Interestingly, *Paecilomyces lilacinus* 1941 isolated from the Red Forest showed positive growth responses in this experiment, but had not in previous experiments (Figs 3–4).

DISCUSSION

The data we have presented strongly suggests that there are significant effects of radioactive emissions on the mean direction of hyphal growth, where the hyphal growth is directed towards the source of ionizing radiation. Of fungal species isolated from radioactively contaminated sites, 86% of the interactions showed positive directional growth towards the source of
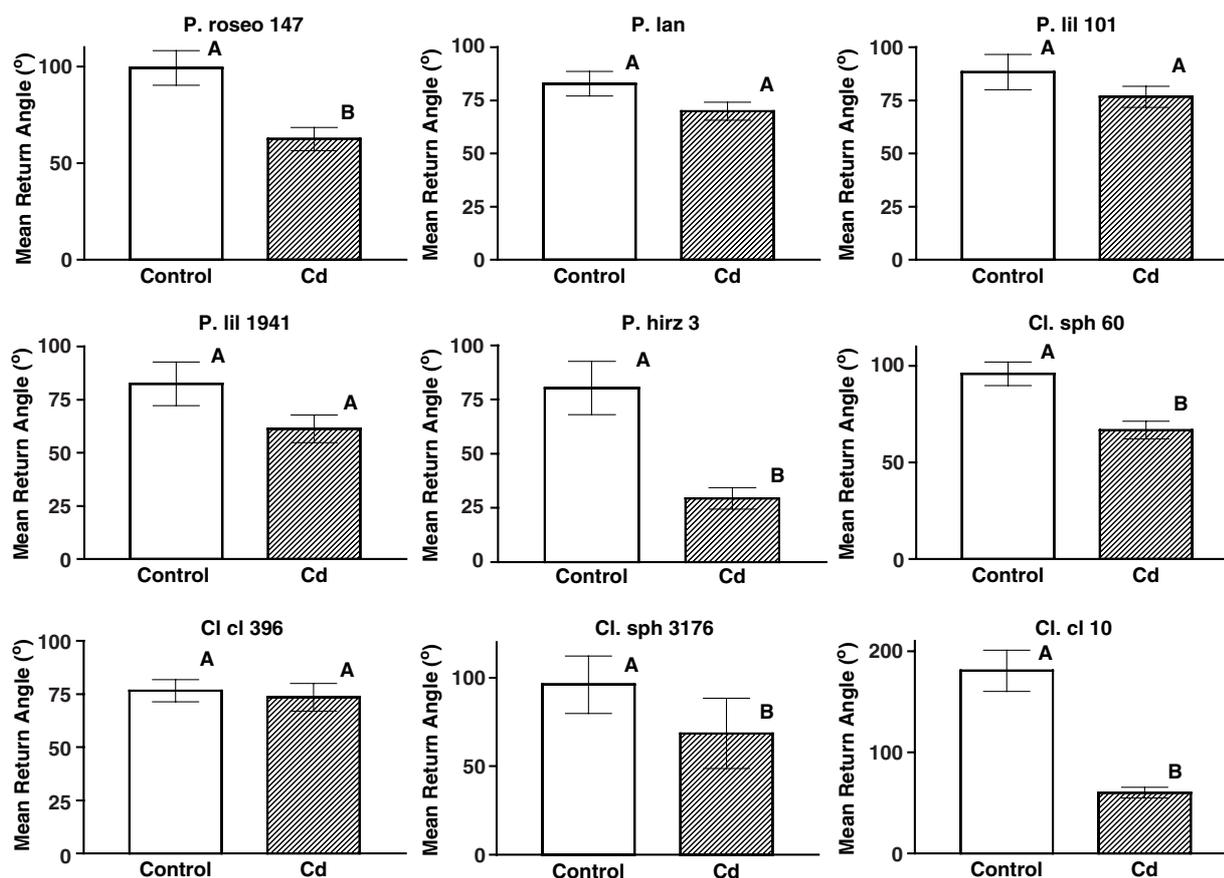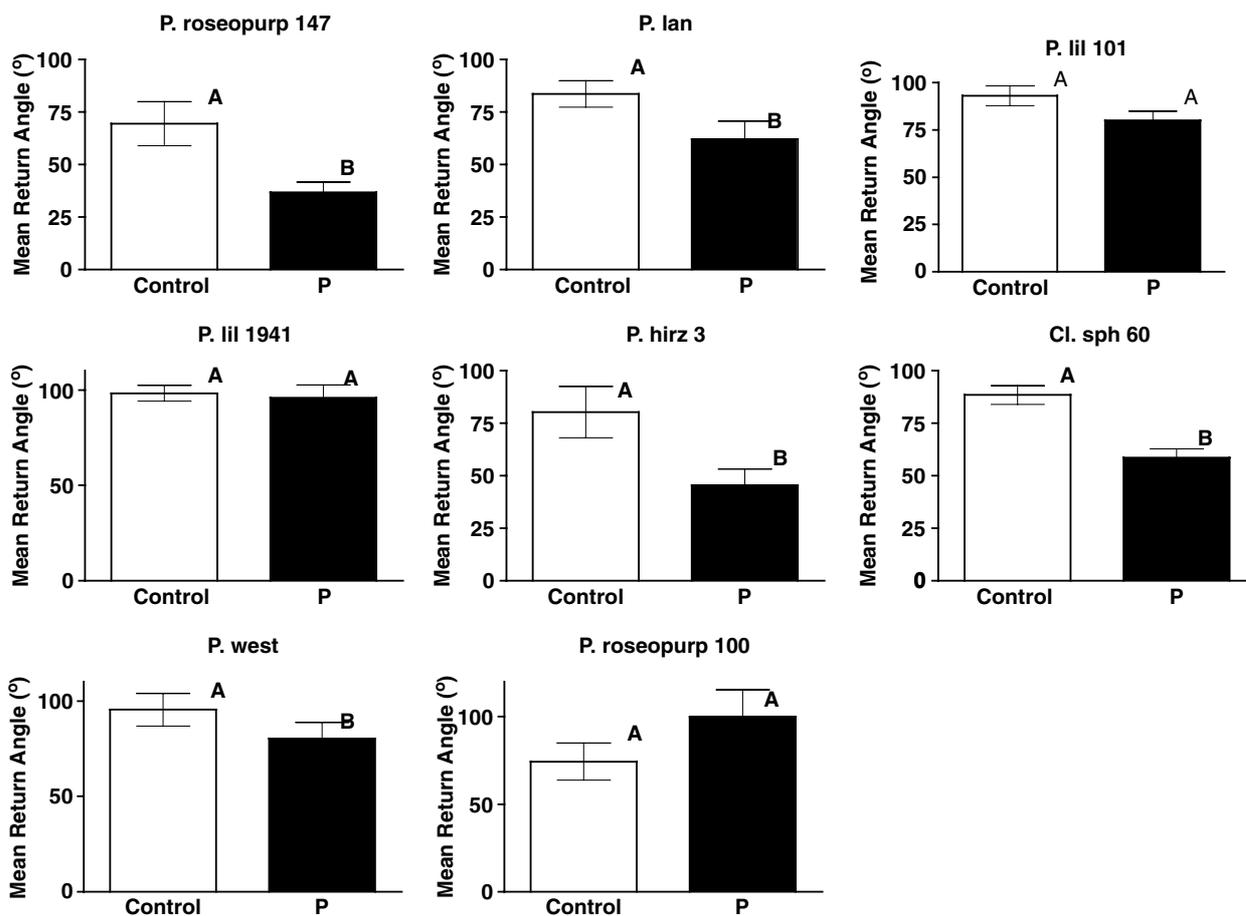
Fig. 3. Mean return angle ($\pm$ SE) of fungi when exposed to a collimated beam of gamma radiation from a [109]Cd source for 24 h (fungal isolates and sources in sequence are *Penicillium roseopurpureum* 147 Red Forest, *P. lanosum* reactor room, *Paecilomyces liliacinus* 101 unpolluted soil, *P. lilacinus* 1941 Red Forest, *Penicillium hirsutum* 3 hot particles, *Cladosporium sphaerospermum* 60 reactor room, *C. cladosporioides* 396 unpolluted soil, *C. sphaerospermum* 3176 unpolluted soil, *C. cladosporioides* 10 reactor room). Adjacent histogram bars bearing the same letter are not significantly different at $P = 0.05$.

ionizing radiation. Evidence has been suggested for the presence of positive radiotropism or directed growth towards the source of radioactivity by a number of fungi (Zhdanova *et al.* 1991, 1994), but this was to carbon-based 'hot particles' where other stimuli may have affected the direction of hyphal growth. In our study, directed growth occurred in the absence of any chemical influence of the source of radioactivity, since the source was physically separated from the fungal culture. In this study, the directional growth has been shown to originate in response to clean beta and gamma emissions. The nature of the ionizing radiation receptor system within the fungal cell is, as yet, unknown. However, it is possible that melanin or other natural quinine pigments in the fungal cell wall could act as this receptor as it has been shown to protect lichenized fungi from UV radiation (Gauslaa & Solhaug 2001), fungi on leaf surfaces (Sundin 2002), and has the capacity to change biochemical pathways in cells when exposed to UV light (Huselton & Hill 1990). Thus, it is possible that melanin or quinines could have a protective effect against ionizing radiation and, by regulating changes in biochemical pathways, could act as a sensor triggering altered growth

characteristics of fungal hyphae. This may explain why there is a greater proportion of melanized fungal species in soils subjected to long-term low levels of ionizing radiation (Zhdanova *et al.* 1995).

Our limited data set of null responses to ionizing radiation of fungi isolated from previously non-radioactive New Jersey soil, together with observational but not quantitative data from earlier studies, suggests that fungi having prior long-term exposure to radiation may have undergone some acclimation to radiation and be more responsive to its presence. It is possible that the increased melanin or quinine content of these exposed fungi may be the mechanism for eliciting a greater directional growth response in these 'pre-adapted' isolates. A more detailed study of the melanin and other pigment content of fungi exposed to ionizing radiation needs to be undertaken.

Although our study provides strong evidence that the direction of growth of emerging fungal hypahe is influenced by the presence of an direction of source of ionizing radiation *per se*, we suggest there may be alternative hypotheses for the observed directional growth response. These may involve: (1) Hyphal cell responses to secondary, low energy, emissions caused

Fig. 4. Mean return angle ($\pm$ SE) of fungi when exposed to a collimated beam of beta radiation from a ^{32}P source for 24 h (fungal isolates and sources in sequence are *Penicillium roseopurpureum* 147 Red Forest, *P. lanosum* reactor room, *Paecilomyces liliacinus* 101 unpolluted soil, *P. lilacinus* 1941 Red Forest, *P. hirsutum* 3 hot particles, *Cladosporium sphaerospermum* 60 reactor room, *P. westlingi* reactor room, *P. roseopurpureum* 100 unpolluted soil). Adjacent histogram bars bearing the same letter are not significantly different at $P = 0.05$.
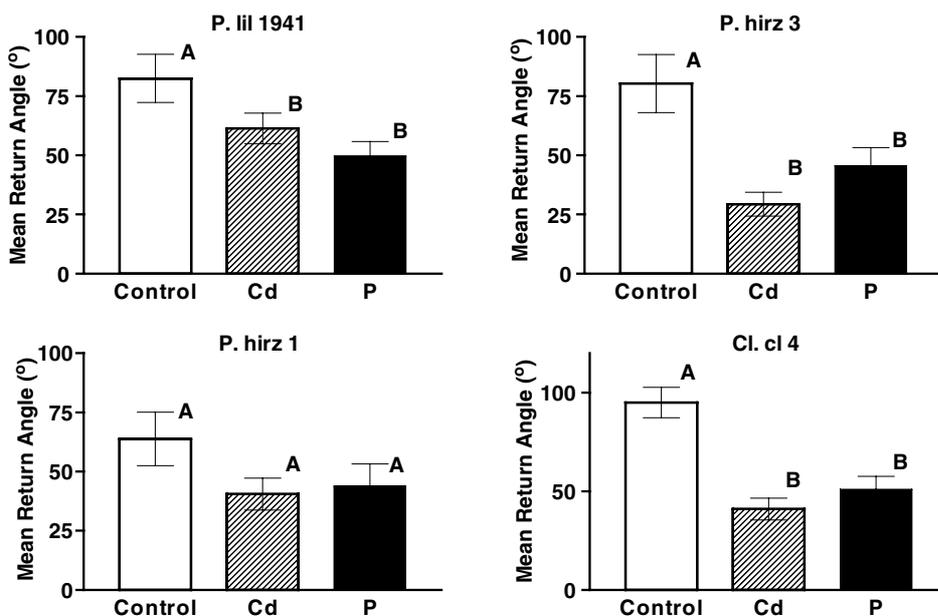
Fig 5. Mean return angle ($\pm$ SE) of fungi when exposed to a collimated beam of gamma radiation from a ^{109}Cd source or beta radiation from a ^{32}P source (fungal isolates and sources in sequence are *Penicillium lilacinus* 1941 Red Forest, *P. hirsutum* 3 hot particles, *P. hirsutum* 1 hot particles, *Cladosporium cladosporioides* 4 soil in 10 km zone). Histogram bars bearing the same letter are not significantly different at $P = 0.05$.

by the primary ionizing radiation, such as light and low energy electrons; (2) hyphal responses to increased temperature caused by ionizing radiation passing through aqueous media; or (3) the use of ionizing radiation as an energy source for metabolic processes.

Support for the first alternative hypothesis comes from preliminary evidence (Yulyia Blazheevskaya, unpubl.), which suggests that low energy white-light stimulated hyphal extension but not directional growth; the effect of secondary low energy electrons has yet to be determined. Ionizing radiation is also known to cause local heating of aqueous media, to which fungi could respond. We calculated the potential heating effect of emissions from our ^{32}P source (2.065×10^8 Bq) by assuming a target volume of water of 0.01 ml. The target volume temperature would increase by approximately 0.003 $^\circ$C d^{-1}, without accounting for heat dissipation. We cannot be certain, but doubt, if this small change in temperature could be sensed by the fungal hyphae and influence their directional growth. Finally, the hypothesis that the fungal hyphae are able to use the energy from ionizing radiation directly into biochemical pathways, using a hitherto unknown, highly sensitive receptor system, is yet to be evaluated. Adoption of this last hypothesis would require evidence for a suitable energy receptor and metabolic pathway to utilize this energy. Given that fungi are regarded only as heterotrophs, their ability to act as autotrophs without a symbiotic partner would alter our understanding their role in the ecosystem.

This newly discovered property of directional growth of fungal hyphae towards ionising radiation may be functionally significant. Some members of the microfungal saprotrophic community have been shown to grow towards and decompose radionuclide-containing organic debris. What is less well-known is if these fungi then release the inorganic form of the radionuclide back into the environment, due to the comparatively short turnover time of these fungi, or if they are capable of more long-term radionuclide immobilization, as has been suggested for mushroom-forming ectomycorrhizal fungi (Haselwandter 1978, Dighton & Horrill 1988, Murumatsu *et al.* 1991, Haselwandter & Berreck 1994, Zhdanova *et al.* 1994). The nature of the radiation source to which these fungi can respond is still largely unknown, although our studies suggest responses to beta and gamma radiation and that these fungi may be sensitive to changes in radiation dose (data not shown). However, for these fungi to be involved in the decomposition of highly radioactive materials, they must be resistant to the ionising radiation (Mironenko *et al.* 2000). The ecophysiological interactions between low level ionising radiation, behaviour, and the functioning of fungal communities is largely unknown. Unravelling these complex interactions may be important in allowing us to understand the potential role of fungi in determining the fate of radionuclide pollutants in the environment, and the potential and actual roles of fungi in site remediation.

Further research is needed to evaluate the ability of these fungi to utilize energy from ionising radiation, the effects of long-term, low-dose radiation on the genetics and gene expression of these fungi could lead to clues of the role of these fungi in the decomposition of radionuclide containing materials in the environment and their abilities to sequester radionuclides into their own biomass.

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