DIVERSITY OF CHYTRIDIACEOUS FUNGI IN A CROPPING SOIL

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Abstract

This pilot investigation found that cropping had an insignificant impact on the diversity of chytridiaceous fungi at an experimental site in semi-arid northern NSW, Australia. A total of 14 different chytrids were observed on baits placed with soil. Ten different fungi were found in soil from each of a vegetated adjacent block, and a cotton/wheat rotation, eight fungi were found under each of a cotton/cotton and cotton/vetch rotations, and six under a fallow (no plants) treatment. Six fungi were common to each treatment. One fungus was only found at the adjacent site, three others were only found under the rotations. The data indicate that diversity of chytridiaceous fungi and plants were unrelated at the experimental site.

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Introduction

Chytridiaceous fungi, herein called chytrids (Barr 2001), have been collected from various environments including sphagnum bogs (Sparrow & Lange 1977), beaches and dunes (Booth 1971a, 1971b), halomorphic soils (Booth 1969), and drought affected soils (Willoughby 1984, Willoughby & Rigg 1983). Chytrids are probably ubiquitous in soil (Longcore 2001).

The nutrition of chytrids is not well understood (Powell 1993). Experiments in axenic culture (Barr 2001), and use of diverse baits to trap different species, indicate that a diversity of substrates may be used by chytrids. Thus we might predict that a large array of different carbon-based substrates in soil would support a high diversity of chytrids. For instance, diverse plant populations might sustain diverse populations of chytrids as happens for other fungi (Apinis 1958, Christensen & Whittingham 1965, Wicklow *et al.* 1974) while relatively high concentrations of organic matter might support abundant populations of chytrids (Lee 2000). The diversity of chytrids under heath vegetation in the Sydney basin was less than under nearby dry sclerophyll, wet sclerophyll and rainforest vegetation (Letcher, McGee & Powell 2004), possibly due to the periodically dry, mineral deficient environment. Thus the factors that determine diversity of chytrids in cultivated soils might be unrelated to the diversity of the plants growing in the soil.

Chytrids form zoospores which require free water for dispersal. Chytrids may rapidly pass from stage to stage of their life cycle, with the total time as short as 48 hours (Ward 1939). In ecological terms, such fungi may be thought of as ruderal (though see Andrews 1992) in that the size of populations may increase rapidly when nutrients are available. If soil chytrids follow this pattern, then their populations may markedly fluctuate in soil used for cropping. Chytrids might be relatively abundant during periods of 'recovery' from disturbance such as flooding and cultivation, and depleted during extended periods of plant growth when other fungi are more competitive.

Cultivation of soil is associated with loss of carbon, and changes in plant exudates and organic matter (Ayanaba *et al.* 1976, Ellert & Gregorich 1996, Srivastava & Singh 1989). Cropping reduces the diversity of plants and plant remains. Decreases in carbon stores in cropping soil are attributed to inputs of carbon being reduced, and rates of decay of

plant litter being enhanced. The effect of cropping on chytrids is unknown. Disturbance, especially cultivation, often increases the concentration of biologically available nutrients in soil which may lead to a temporary increase in abundance of chytrids (Lozupone & Klein 2002). However, a reduction of chytrid abundance and diversity is predicted for soils in which cropping is long-standing.

The present study investigated the diversity of chytrids in an agricultural soil. The objective was to determine whether chytrid diversity is directly associated with plant diversity. If the prediction is supported then the diversity of chytrids will be highest in soils where many annual and perennial plants are growing together, followed by cropped soils with simple rotations, and least diverse in long term fallow where few plants grow.

Materials and Methods

Soil samples were collected from replicated plots of the experimental field at the Australian Cotton Research Institute, Narrabri, NSW, Australia (ACRI:149°47'E 30°20'S). Rotations in place for more than three years include cotton/cotton (cotton in summer, fallow in winter), cotton/wheat (cotton in summer, wheat in winter), cotton/vetch (cotton in summer, vetch in winter), and fallow (without plants). Soil was also collected from plots set out on an uncultivated adjacent site located less than 100 m from the experimental field. The adjacent site had a cover of annual and perennial grasses and herbs, and a few shrubs. Soil samples were also collected on one occasion from a normally moist site located beside the Namoi River in Narrabri (river site), about 10 km southeast of the ACRI.

Cotton (*Gossypium hirsutum* L.) was sown on 3 October 2002 in the cotton beds of the rotation experiment. All crop rotations were irrigated and the adjacent and river sites were not irrigated. Cotton and adjacent plots were defoliated on 31 March 2003 and again on 7 April 2003. Cotton lint was harvested from 7 to 15 May 2003. The cotton beds were then cultivated using vertical harrows. Vetch (*Vicia sativa* L., cv. Namoi) and wheat (*Triticum aestivum* L., cv. Yallaroi) were sown on 23 May 2003 into the cotton/vetch and cotton/wheat rotations respectively.

Four or five soil samples (approximately 100 grams each) were collected on 16 December 2002 at each of three depths (surface, 5 cm and 10 cm) from each of five replicate plots of all of the rotations and the adjacent site to determine if soil depth affected the presence of chytrids. Subsequently, five soil samples were collected at less than 10 cm depth from all plots (total 25) in each of the rotations and the adjacent site on 11 March 2003, 2 May 2003 and 17 June 2003. Samples were collected from the fields prior to irrigation to reduce the effect of flooding on chytrids. No samples were taken from the cotton/vetch rotation in March. The river site was only sampled in March to provide a check on diversity in undisturbed soils, and to indicate whether the common species are widespread in the district. Soil samples were returned to the laboratory immediately, and stored at 4°C until processed.

Ten g of each soil sample was placed in a separate Petri dish and 40 ml of sterile deionized water added. The soil was briefly stirred. Baits of sterile pine pollen, keratin (snake skin), chitin (prawn exoskeleton) and cellulose (onion leaf epidermis) were floated on the surface of the water and the Petri dish lid replaced. Baits were examined microscopically after six and 12 days (Barr 1969).

Identifying any particular species requires multiple observations over a period of several days to weeks in order to observe all the characters needed to identify each fungus (Letcher & Powell 2002b). For the present study, chytrids were grouped based on morphology, and then, where possible, chytrids were isolated to pure culture on YpSs (yeast, peptone and soluble starch), PYG (yeast, peptone and glucose), PmTG (peptone, tryptone and glucose) or cellulose medium, and their cultural characteristics used to tentatively identify the fungi (Karling 1977, Sparrow 1960).

Diversity of chytrids was calculated for each field site using the Gleason index (Burnett 2003) and again using the Simpson index (Simpson 1949). Abundance is notoriously difficult to estimate. Here, we used the proportion of pollen grains colonised by chytrids, or visually estimated as the proportion of cellulose, chitin or keratin substrate occupied by sporangia to indicate abundance.

The data on diversity in this pilot study were both skewed and kurtosed. The samples over time were from within one field indicating a lack of independence. Thus the nonparametric Mann-Whitney was used to compare diversity under each type of vegetative cover using data for each harvest as the sample (Zar 1996). The Sign test was used to compare presence and absence of fungi under each type of vegetative cover (Freund & Simon 1992).

Results

Of the 14 chytrids identified at the ACRI (Fig. 1), a total of ten different chytrids was found in the soil

Table 1. Frequency of collection, and Simpson's Index and Gleason Index of chytrids at ACRI. Co/Co – cotton/cotton rotation, Co/W – cotton/wheat rotations, Co/V – cotton/vetch rotation, fallow (Fal) and adjacent (Adj) site, with the (n) number of samples for each site. * Unable to collect samples March 2003, # single collection.

Fungus	Co/Co	Co/W	Co/V*	Fal	Adj	Total number of sites	Namoi River#
	(90)	(87)	(62)	(90)	(90)		(15)
Rhizophlyctis rosea	3	4	3	3	4	5	1
***Catenophlyctis* sp.**	4	4	3	4	3	5	1
Unknown C	3	4	2	3	3	5	0
Rhizophydium subglobosum	0	0	2	0	1	2	1
Rhizophydium sphaerotheca	4	4	3	4	4	5	0
Rhizophydium pollinis-pini	4	4	3	4	4	5	1
Unknown G	0	0	0	0	3	1	0
Unknown H	1	3	0	0	0	2	0
Unknown I	0	0	1	0	0	1	1
***Rhizophydium* sp. parasite 1**	0	1	0	0	0	1	1
***Rhizophydium* sp. parasite 2**	0	1	0	0	0	1	0
***Catenophlyctis* sp.**	1	0	0	0	1	2	0
***Allomyces* sp. 1**	1	1	2	4	2	5	1
***Allomyces* sp. 2**	0	1	0	0	1	2	0
Others							3
Total	8	10	8	6	10		10
H_S	0.54	0.65	0.57	0.45	0.74		
H_G	1.11	1.79	1.45	1.11	1.77		3.32

collected from the adjacent site and the cotton/wheat rotation, eight from cotton/vetch rotation, and the cotton/cotton rotation and six from the fallow soil (Table 1). Six chytrids were present at all sites. One chytrid was only found at the adjacent site (Table 1). While most chytrids were seen in the first sampling, species' richness increased over time in soil from the cotton/wheat rotation (five to ten), and in the fallow plots (five and six, Table 2). Two chytrids are parasites on other chytrids and both were uncommon. Because the richness of chytrids was similar at each depth in the first collection of samples, a single sample was collected subsequently. Ten chytrids were obtained from the river site, of which four were common at the ACRI, and three were not found at ACRI (Table 1). Analysis of data using the Mann-Whitney test indicates that species richness did not differ significantly between sites at ACRI ($P > 0.05$). The presence of chytrids found associated with each type of vegetative cover did not differ significantly between sites or between the river and ACRI (Sign test, $P > 0.05$).

The two indices used to indicate diversity showed a similar trend. The Simpson indices (H_S) (Table 1) indicate the highest chytrid diversity is found at the adjacent site at the ACRI, followed by the cotton/wheat rotation, cotton/vetch rotation, cotton/cotton rotation, and the fallow rotation as the least diverse. The Simpson index is suitable for large sample sizes ($N > 80$) (Burnett 2003) and so is not useful for measuring diversity at the river site ($N = 15$). The Gleason index (H_G) is a simpler measure of diversity, less sensitive to sample size (Burnett 2003). The H_G of chytrid diversity at the river site was well above that of any of the ACRI sites, where the fallow and the cotton/cotton rotation were the least diverse sites (Table 1).

The abundance of the six common fungi at ACRI (Table 3) is presented because insufficient data are available for the remainder. Only *Catenophlyctis* sp. was abundant. Abundance of all other chytrids was low at all times (Table 3). *Rhizophydium pollinis-pini* was present at all sites at ACRI and found on 16% of pollen from the river site in March. Abundance of *Rhizophlyctis rosea* was low and infrequent at all sites at ACRI, and relatively high at the river site

Table 2. Number (cumulative number) of chytrids isolated from soil under each rotation, and the adjacent site at ACRI, and the river site at four times. * = no sample collected.

	Mixed Plants	Cotton Cotton	Cotton Wheat	Cotton Vetch	Fallow	River
December 2002	9	6	5	7	5	*
March 2003	6 (10)	4 (7)	9 (9)	*	5 (5)	10
May 2003	5 (10)	6 (8)	6 (9)	7 (8)	6 (6)	*
June 2003	6 (10)	5 (8)	8 (10)	5 (8)	6 (6)	*

Table 3. Mean (± SD) abundance of common chytrid morphotypes in soil from the rotations at the ACRI.

		Co/Co	Co/W	Co/V	Fallow	Adjacent
Morphotype	**Bait**					
Unknown C	**Pollen**	2 ± 2	2.4 ± 0.8	2.2 ± 1	2.8 ± 2.5	0.5 ± 0.4
Rhizophydium sphaerotheca	**Pollen**	2.2 ± 2	1.6 ± 1.1	1.5 ± 1	1.5 ± 0.8	0.9 ± 0.3
Rhizophydium pollinis-pini	**Pollen**	3.9 ± 4.8	1.6 ± 0.8	1.5 ± 1.1	0.9 ± 0.3	1.5 ± 0.7
Rhizophlyctis rosea	**Onion**	0.8 ± 1	2.8 ± 4.2	2.3 ± 2.8	0.5 ± 0.6	3.9 ± 3.1
***Catenophlyctis* sp.**	**Keratin, chitin**	12.3 ± 2.9	4.4 ± 4.2	13.7 ± 6.1	18.8 ± 10	1.9 ± 1.9
***Allomyces* sp.**	**Keratin, chitin**	0.8 ± 1.5	0.8 ± 1.5	0.8 ± 1.2	0.5 ± 0	1 ± 1.4

where it colonized 22% of the onion skin.

Discussion

A total of six to ten chytrid morphotypes were found in the soils collected on four occasions from plots in five sites at the ACRI over a period of seven months. In addition, limited sampling from the river site resulted in ten morphotypes, the same as the adjacent site. Though diversity indices indicated differences, these differences are not statistically significant and our hypothesis of a decline in diversity with reduced plant diversity is rejected. Cropping appears to have no statistically significant impact in diversity of chytrids at ACRI.

Using a similar sampling process, 14 different chytrids were observed in open heath, and 23 under dry sclerophyll vegetation of the Sydney Basin of eastern Australia (Letcher *et al.* 2004). While the number of chytrids in the heath appears to be similar to the adjacent and river sites, the dry sclerophyll vegetation had a significantly greater diversity. Letcher *et al.* (2004) observed 14 out of 34 chytrids on pollen, which is similar to the ratio found at the ACRI, indicating that our observations are comparable. Overall, limited chytrid diversity was observed at the ACRI and in heath vegetation. Thus it might be argued that a factor other than plant diversity limits overall diversity of chytrids.

While more chytrids were found in soil from the adjacent than the fallow site, similar numbers were found in rotations with severely reduced plant diversity. Interestingly, two parasitic chytrids were found in cultivated soils used in the cotton/wheat rotation. If the parasites are removed from calculations, the cotton/wheat rotation has eight chytrids, the same as the other two rotations. Six chytrids were common to all sites at the ACRI. One chytrid was relatively abundant at the rotation sites and absent in the adjacent site. The remaining chytrids were rare. Thus ten chytrids were isolated from each relatively undisturbed site, eight from cropped soils, and six from the fallow site. While the trend is towards lower diversity of chytrids with fewer plants, six chytrids were found at all sites.

The inclusion of the fallow site provides a simple test of the importance of plant materials for growth and survival of chytrids. The fallow site has no crop cover, though it may have had the occasional weed over the last three years. Some insects may have shed exoskeletons, and fungi no doubt continue to slowly grow during periods when the soil is moist. In other words, limited carbon continues to be deposited on and in the soil. However, these substrates would be rare and possibly localised at the soil surface. Six common morphotypes were found at all sites at the ACRI.

The substrates used by these chytrids in the field remain unclear. The fallow site has extremely low concentrations of organic carbon and the carbon is in a form that is thought to be complex and resistant to

degradation (Hulagalle 2000). The data support the possibility that some widespread chytrids may degrade complex carbon substrates in nature or use a diversity of widespread substrates for growth and development.

Alternatively, the fungi may be present in soil as resistant structures that germinate in baited solutions. If the fungi are present as resistant structures in soil, and the structures germinate during the baiting procedure, then reasons for remaining dormant require elucidation.

In this investigation four different baits resulted in different arrays of chytrids being observed. Presumably different substrates are important for specific taxa of chytrids in nature. The limited diversity of chytrids observed may be due to use of inappropriate baits for the chytrids present at the ACRI.

Similarly, we observed one abundant chytrid. The technique we used is only indicative. The coverage of baits may indicate an increased proportion of zoospores settling on the bait, or the rapid release of zoospores from few sporangia, rather than the relative number of sporangia present in soil. *Catenophlyctis* sp. survives high temperatures when dry (Gleason, Letcher & McGee 2004) and has other cultural characteristics that appear important for growth at Narrabri (Gleason, Letcher & McGee unpublished). Thus the relative abundance of the chytrid may be ecologically important.

The primary source of organic energy in soil arises from photosynthesis. Thus many heterotrophic organisms, including fungi, are closely linked to plants. *Catenophlyctis* sp. was slightly more abundant in soil from the fallow than the vegetated plots indicating a negative relationship between the chytrid and plants. Amongst many factors, the size of populations of chytrids is likely to be related to the presence and availability of food, and competition with other microbes for that food. In addition, plants or plant remains may release to the environment compounds that are toxic to some microbes. Thus the relation between plants and chytrids may be complex. These hypotheses can be tested in controlled experimental conditions.

Possible explanations for low overall diversity are unclear. Letcher & Powell (2001, 2002a) observed more chytrids under mosses where presumably water is sequestered for extended periods and is more evenly distributed through the soil profile. Thus moist sites might yield greater diversity of chytrids, including those susceptible to desiccation. Soils from the river site are moist for much of the year. The rotation soils are irrigated and their moisture content is kept at levels to maximise plant growth. The adjacent site is not irrigated. The climate is naturally hot and dry. A similar diversity of chytrids in soil from the river, adjacent and the cotton/wheat rotation sites does not support a hypothesis of available moisture being related to chytrid diversity, but this lack of relationship may be due to other climatic factors such as heat (Gleason *et al.* 2004) and associated desiccation.

The ACRI is located in the hot, dry, semi-arid part of northern NSW, Australia, where heat and lack of water may have an overriding importance in determining chytrid diversity. The dissemination of chytrids in nature is unclear, though data on survival of some chytrids indicate that they may be transported with dust (Gleason *et al.* 2004). Thus settled areas may have chytrids of exotic origin adapted to the current conditions in addition to indigenous species adapted to the original conditions. With this in mind it is interesting to note that one chytrid was only found at the adjacent site, and then in three of the four collections. A further three fungi were only found at the river site. These data support the view that some chytrids have specific conditions regulating their survival, and that any one location may have a limited diversity under hot, dry, semi-arid environments. These conditions remain to be elucidated.

A limited diversity of chytrids was found at the ACRI. Though plant and fungal diversity appear to be only weakly correlated, the case for plants reducing the size of populations of some chytrids is also supported. Indeed, chytrids were found where there have been no plants for the last three years, indicating that some chytrids may use complex organic substrates. While it is clear that chytrids are present in soil from hot dry semi-arid regions, the mechanisms regulating their presence and function are, as yet, unclear.

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