

## The effect of ecological disturbance on competition between *Crinipellis pernicioso* and other tropical fungi

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### Synopsis

Mycelia of the tropical fungus *Crinipellis pernicioso* obtained from cocoa and lianas in Ecuador were grown on media ranging from  $-0.45$  MPa to  $-9.0$  MPa water potential and their responses compared to those of other agarics and *Xylaria* species isolated from cocoa. Antagonism between mycelia of *C. pernicioso* and other isolates was also assessed over the same range of water potentials. It is hypothesised that *C. pernicioso* and other canopy inhabiting fungi of tropical forests are adapted to water stress, but are not competitive with fungi which colonise later, following litterfall, from lower litter and soil horizons, where water availability is higher. *Xylaria* spp. proved to be aggressive antagonists of *C. pernicioso* over a range of water potentials.

### Introduction

Ecologists have emphasised the storied structure of tropical forests and the dependence of epiphytic communities. Hedger (1985) considered that species composition of fungal communities of saprotrophic agaric fungi is distinctly different from that of the litter and soil horizons in tropical forest. He proposed that these agarics show adaptations to the daily and seasonal fluctuations in the water availability which occur in the canopy of most tropical forests.

In the study reported here we have used the Witches' Broom Disease fungus, *Crinipellis pernicioso* (Stahel) Singer, as a possible example of a canopy-inhabiting fungus. The biology and ecology of this fungus, which occurs in Amazonia and other tropical areas of South America, is reviewed elsewhere (Evans 1981; Rocha & Hedger *et al.* 1987). It occurs both on cocoa (C-type) and on liana and other plants in the forest (L-type). Infection of the canopy of cocoa by the basidiospores of the fungus causes the development of large brooms in which, after death, a saprotrophic mycelium utilises the lignocellulose resource to produce flushes of basidiomes in wet seasons. Optimum basidiome production has been shown by Rocha & Wheeler (1981) to be dependent on daily fluctuations in the water content of the brooms. Gard (1986), in researching the relationship between humidity and basidiome production by *C. pernicioso* in Rondonia, Brazil, found that brooms on the litter which stayed wet for six hours a day longer than those in the canopy and produced significantly fewer basidiomes. Hedger (1985, 1988) also showed, in Ecuador, that basidiomes incorporated into the litter beneath cocoa soon ceased to produce basidiomes, and also found that the mycelium of *C. pernicioso* could not be recovered from basidiomes buried for six months in the F1 layer of the litter. However, it persisted for up to three years in brooms suspended in the canopy.

These observations indicate that the ecological disturbance, and associated change in microclimatic conditions, of fall of brooms from canopy to litter may alter the

competitive balance between *C. perniciosus* and other broom-colonising fungi. In order to explore this possibility we have investigated interactions, over a range of water potentials, between the mycelium of *C. perniciosus* and other fungi isolated from witches' brooms. This model may represent the possible changes in water content which take place when brooms fall from the canopy into the litter layer.

## Materials and methods

### Isolation of fungi

Witches' brooms of cocoa were imported in the U.K. from Ecuador under licence and kept in cabinets under conditions similar to those described by Suarez (1977) and Rocha (1983), that is, 25–27°C with alternate 8 h, 16 h wet/dry cycles using a humidifier. Agarics which sporulated on the brooms were isolated by allowing basidiospores to deposit from basidiomes suspended over Potato Dextrose Agar (PDA) in petri dishes held at 25°C overnight. Resultant polyspore cultures were transferred to PDA slopes at 25°C. Cultures of *Xylaria* and *Nodulisporium gregarium* (Berk. & Curt.) Meyer were obtained by surface sterilising either 1.0 cm length broom sections, or 1.0 cm length ascocarp sections, for five minutes in 1% NaOCl solution, followed by washing in sterile distilled water, plating on PDA containing 0.05% Streptomycin at 25°C and subculturing from the margin of colonies after three to five days incubation. Cultures and dried material were deposited in the Herbarium, UCW Aberystwyth.

### Response of mycelia to different water potentials

Isolates were grown on a basal medium of Glucose 2%, Malt Extract 2%, Peptone 0.1% and agar 1.5%, in 1 l of distilled water. Potassium chloride was added to the medium before autoclaving to achieve a range of water potentials from –0.45 MPa to –13.8 MPa (Robinson & Stokes 1959). Growth rate was quantified in 9 cm petri dishes with medium inoculated centrally with 6 mm agar discs cut with a sterile cork borer from the margin of a colony, actively growing at 25°C on PDA agar. Mycelial extension was measured at 24 h intervals along three axes from the centre of the colony for up to one month. Petri dishes were kept in plastic bags, to prevent water loss.

Interactions at different water potentials were assessed by dual inoculation of plates with 6 mm discs placed 2.5 cm apart. Growth along three axes measured toward the confronting colony margin was compared with growth on the opposite side (Webber & Hedger 1985). In all cases, five replicate plates were prepared for each experimental condition.

### Isolation of fungi

The following taxa were used for study:

**Agaricales:** *Crinipellis perniciosus*, *Mycena theobromicola* (Murr.) Dennis, *Hohenbuehelia barbatula* (Berk. & Curt.) Dennis, *Clitopilus rhodotrampa* Sing., *Crepidotus cuneiformis* Pat., *Coprinus jamaicensis* Murr., *Pluteus* sp., *Melanotus alpiniae* (Berk.) Pilát.

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**Sphaeriales:** *Xylaria grammica* (Mont.) Fr., *Xylaria multiplex* (Kze. ap. Fr.) Fr. complex Berk. & Curt., *Xylaria* sp. 12, *Xylaria* sp. 59, *Xylaria* sp. 118.

**Hyphomycetales:** *Nodulisporium gregarium*.

All the taxa were isolated from carpophores on witches' brooms or plated out on witches' broom sections, with the exception of *Xylaria multiplex*, *X. grammica* and *Xylaria* 118, which were isolated from ascocarps growing on dead wood in cocoa litter. Each taxon was represented by only one isolate.

## Results

### Comparison of growth rates of isolates at different water potentials

Cultures of *C. perniciosus* obtained from witches' brooms from Ecuador (C-type) proved to be tolerant of water stress, being able to develop small colonies at levels as low as  $-8.0$  MPa. Cultures obtained from basidiomes on liana (L-type), also from Ecuador, behaved similarly (see Fig. 1). Both grew optimally at  $-0.45$  MPa. The even other agarics isolated from witches' brooms proved less tolerant of water stress and none grew at  $-4.4$  MPa. *Mycena theobromicola* seemed particularly intolerant, with no growth detected at  $-3.1$  MPa even after several months incubation at  $25^{\circ}\text{C}$  (Fig. 1).

In contrast, isolates from *Xylaria*, or with a possible affinity as an anamorph in the case of *Nodulisporium gregarium*, showed tolerance to water stress (Fig. 2). In some isolates, growth at  $-0.9$  MPa was equal to or slightly greater than at  $-0.45$  MPa, and some growth was recorded at  $-9.0$  MPa for most of the isolates, although, as with *C. perniciosus*, there was a long lag phase before growth occurred at this water potential.

### The effects of water potential on interactions between isolates and *C. perniciosus*

The outcome of confrontation between *C. perniciosus* colonies and other agarics was clearly influenced by the water potential of the medium. At  $-0.45$  MPa in confrontations between other agarics and *C. perniciosus*, mycelial extension of both colonies ceased on contact, either accompanied by the formation of a pigment along the contact line (type 1 reaction) or by a clear zone between the two colonies (type 2 reaction). At lower water potentials ( $-0.9$  MPa and  $-1.3$  MPa), interactions changed from type 1 to type 2, with a decreased effect on the rate of *C. perniciosus* mycelial growth on the side towards the opposing colony. *Coprinus jamaicensis* is an example (see Fig. 3, bottom). Except for *C. jamaicensis*, the isolates tested grew so slowly at  $-3.1$  MPa that no contact occurred, and there was no effect on the *C. perniciosus* colony (Table 1).

In contrast, the *Xylaria* and *Nodulisporium gregarium* isolates proved to be aggressive invaders of *C. perniciosus* colonies over a range of water potentials (Table 2). Following contact, five of the isolates invaded the *C. perniciosus* colony more rapidly at  $-1.3$  MPa than at  $-0.45$  and  $-0.9$ , although at  $-3.1$  MPa and below there was a decrease in the amount of penetration of the *C. perniciosus* colony by the antagonists (see Table 2 and Fig. 3, top). *Xylaria grammica* was the only species which penetrated the *C. perniciosus* colony at  $-7.1$  MPa.

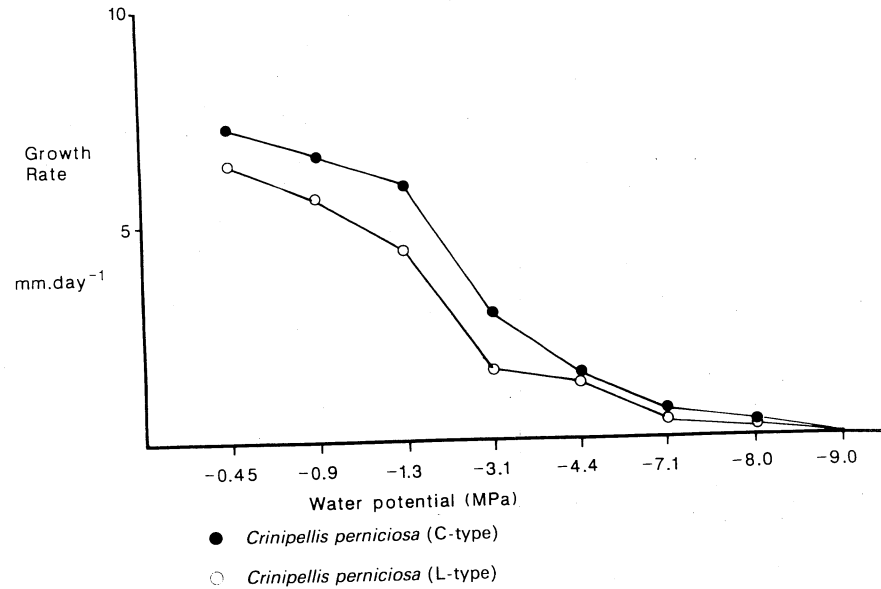
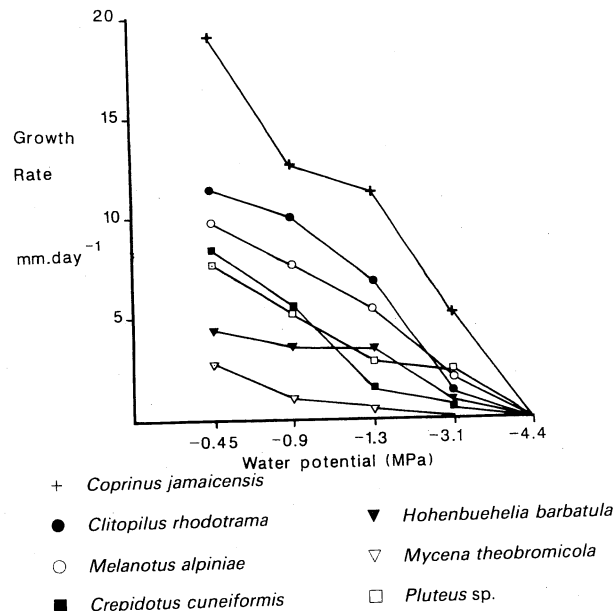


Figure 2



Table

Figure 1. The effect of water potentials on the growth rates of isolates of *C. pernicioso* (C & L types) (upper figure) and seven other agarics (lower figure).

Typically the growth form of the mycelium of these antagonists changed on contact with the *C. pernicioso* colony. In most cases penetration of the colony was accompanied by vigorous formation of mycelial cords and fans, usually unpigmented, which were less developed in areas of the colony outside the sector occupying the *C. pernicioso* colony (Fig. 3, top). The cord forming sector often extended more quickly than the other parts of the colony, and rapidly covered the entire surface of

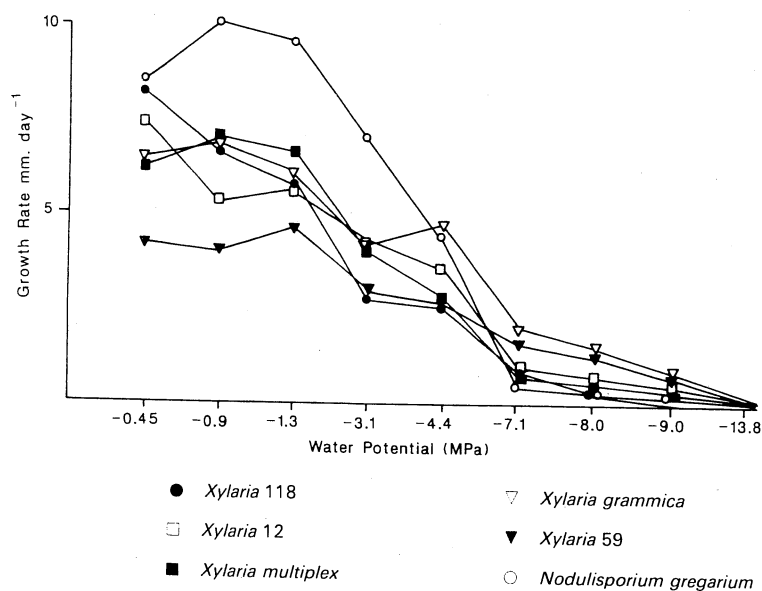
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2. The effect of water potentials on the growth rates of five isolates of *Xylaria* and *Nodulisporium gregarium*.

1. Effect of water potential on interactions between *Crinipellis perniciosus* and other agarics isolated from witches' brooms.

Isolate	Water potential (MPa)				
	-0.45	-0.9	-1.3	-3.1	-4.4
<i>Coprinus jamaicensis</i>	1	1	1	2	4
<i>Crepidotus cuneiformis</i>	2	2	2	3	4
<i>Clitopilus rhodotrampa</i>	1	2	2	3	4
<i>Hohenbuehelia barbatula</i>	1	2	2	3	4
<i>Melanotus alpiniae</i>	1	1	2	3	4
<i>Mycena theobromicola</i>	1	2	2	4	4
<i>Pluteus</i> sp.	2	2	2	3	4

Interaction Types (after one month's growth at 25°C):

- (1) deadlock, with formation of pigment at the confrontation line;
- (2) deadlock, with a clear zone of lysed mycelium at the confrontation line;
- (3) no contact;
- (4) no growth of antagonist.

*perniciosus* mycelium. In three isolates, at -0.45 MPa, however, only about 2% the *C. perniciosus* colony area was occupied, followed by cessation of growth and purple pigment formation in the contact zone. This variation in antagonism in relation to the water potential of the mycelium is listed in Table 2.

### Discussion

*perniciosus* proved to be a water stress tolerant fungus, in comparison with the other agarics studied. In general, litter and wood colonising Basidiomycotina have

**Table 2.** The effect of water potential on interactions between *C. pernicioso* and *Xylaria* and *Nodulisporium*.

Organism	Water potential (MPa)					
	-0.45	-0.9	-1.3	-3.1	-4.4	-7.1
<i>Xylaria</i> 12	246	256	356	356	356	57
<i>Xylaria</i> 59	346	346	346	346	34	7
<i>Xylaria</i> 118	26	26	26	26	47	7
<i>X. grammica</i>	346	36	36	3	3	1
<i>X. multiplex</i>	346	346	346	36	36	7
<i>Nodulisporium</i> <i>gregarium</i>	2	36	36	3	3	7

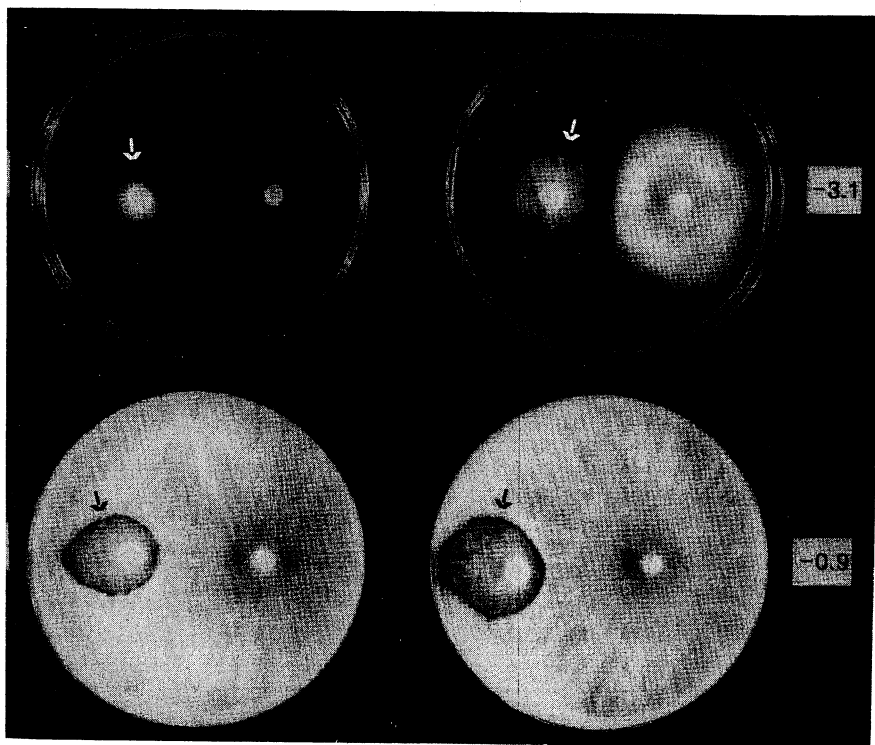
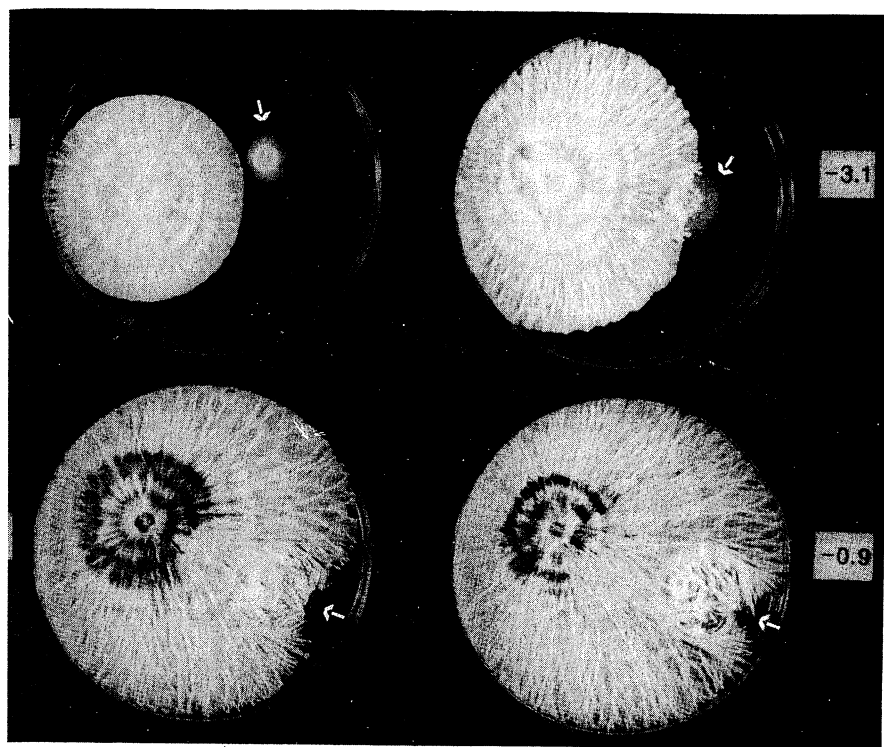
Interaction Types (after 1 month's growth at 25°C):

- (1) deadlock (no penetration of *C. pernicioso* colony);
- (2) partial invasion of *C. pernicioso* colony;
- (3) total invasion of *C. pernicioso* colony;
- (4) inhibition at distance of *C. pernicioso* growth before contact;
- (5) growth stimulation of test fungus mycelium before contact;
- (6) cord formation in *C. pernicioso* colony;
- (7) no contact.

been found to be intolerant of low water potentials (Boddy 1983; Dix 1984; Dix & Frankland 1987), although studies have been confined to temperate species. In the case of *C. pernicioso*, the ability to continue growth at a low water potential must be an advantage in the seasonal and daily fluctuations in water stress in brooms in the cocoa canopy or in suspended liana debris. However, the alleviation of low water stress and the enrichment disturbance caused by the fall of brooms or liana debris into the litter layer changes the competitive balance between *C. pernicioso* and other fungi. Although only a limited selection of these fungi was evaluated as antagonists of *C. pernicioso*, the results of confrontation in agar over a range of water potentials show that the seven taxa were competitive only at high water potentials ( $-0.45$  to  $-1.3$  MPa, equivalent to high water contents in brooms and debris). At lower water potentials (less than  $-3.1$  MPa, equivalent to dry conditions in debris or brooms) they made insufficient growth to be effective competitors. The mycelium of *C. pernicioso* can therefore retain lignocellulose resources in the brooms and liana debris in the canopy, but competition by other fungi is likely to increase after the "disturbance event" of the fall into the litter. Observations in the field confirmed that sporulation of the other agarics studied here such as *Mycena theobromicola* was most frequent on wet witches' brooms buried in cocoa litter, although they also occurred much less frequently on canopy brooms during wet periods (Hedger 1985).

In contrast, the *Xylaria* species examined proved to be as tolerant as *C. pernicioso* of low water potentials and to be effective antagonists of this agaric even at  $-4.4$  MPa. Rogers (1979), Boddy *et al.* (1985), and Whalley (1985) all consider that mycelia of temperate Xylariaceae are able to develop in dry conditions and to be more efficient as competitors at lower water potentials, a characteristic also present in the tropical species studied here. These fungi also have the potential to be canopy

**Figure 3.** Appearance of colonies of *C. pernicioso* and antagonists after three weeks' growth at 25°C on media with water potential  $-0.9$ ,  $-1.3$ ,  $-3.1$  and  $-4.4$  MPa; top: confrontation with *Xylaria* isolate 59; bottom: confrontation with *Coprinus jamaicensis*; position of the *C. pernicioso* inoculum is marked with an arrow.



inhabiting and probably are able to compete for resources with *C. perniciosa*, and replace its mycelium, although the latter species is pre-established by an infective phase in witches' brooms.

It can be concluded that the disturbance represented by fall of litter from the canopy of tropical forest into the litter layer beneath marks a transition between dominance of resources by canopy inhabiting fungi, adapted to a water stressed environment, to occupation by communities of less stress adapted fungi.

### Acknowledgments

We thank the British Council for supporting this study, Dr D. Pegler for the identification of *Clitopilus rhodotrampa* and *Melanotus alpiniae*, and Dr Lynne Boddy for much valuable advice. Brooms were imported into the U.K. under licence from the Ministry of Agriculture, Fisheries and Food.

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