# Effect of volatile phylloplane fungal metabolites on the growth of a foliar pathogen

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*Summary.* Volatile fungal metabolites excluded from various leaf-inhabiting microfungi were tested for their mycostatic activity against the growth of *Pestalotiopsis funerea* Desm., a leaf spot pathogen of *Eucalyptus globulus* Labill. Findings reveal that *Trichoderma viride* and *Trichothecium roseum* exhibited significant antagonistic action, however, *Cladosporium herbarum, Nigrospora sphaerica* and *Papulaspora* sp. showed a stimulating effect. A morphophysiological effect was also observed.

Volatile metabolites of fungal origin have been reported to play an important role in the mycostasis phenomenon in the soil and various other habitats[3-8]. Considering the microbial interactions on aerial plant surfaces, there is no reason to neglect the antagonistic role of volatile fungal metabolites on the development of an invading pathogen. Keeping this in view, the present investigation was undertaken to study the effect of excluded volatile metabolites of various leaf inhabiting microfungi on the growth of *Pestalotiopsis funerea* Desm., which causes leaf spot disease of *Eucalyptus globulus* Labill.

*Materials and methods.* Leaf-inhabiting microfungi including *P. funerea* were isolated by using different cultural techniques of Dickinson[9], and pure cultures of them maintained on Czapek-Dox + 0.05% yeast extract agar. The different fungal species were tested by growing them on Czapek-Dox + 0.05% yeast extract agar in Petri dishes (15 ml medium per dish) for a period of 10 days. Each plate was inoculated centrally with an agar disc of 6 mm diameter cut along the margin of a freshly growing culture of antagonist fungus inhabiting the leaf. After incubation, the lid of each dish was replaced by a bottom containing the above medium, inoculated centrally with a 6 mm agar disc of *P. funerea*. The 2 dishes were fixed together with an adhesive tape. The lid of the control plate, which had not been inoculated, was replaced in the same way. Test plates and control plates were set up in triplicate. After 24, 48 and 72 h of incubation, colony diameter of the pathogen was measured and percent inhibition or stimulation in growth was calculated with respect to the control. Data so obtained were analyzed statistically.

Showing percent inhibition in radial colony growth of *P. funerea* for different time intervals in culture by volatiles of some of the leaf inhabiting micro-fungi

| Culture volatile of | Growth inhibition after different time intervals (%) | | | |
|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h |
| *Alternaria humicola* | 12 | 10 | 8 | 8 |
| *A. alternata* | 18 | 21 | 22 | 17 |
| *Aspergillus candidus* | 0 | 0 | 0 | 0 |
| *A. chevalieri* | 28 | 26 | 26 | 22 |
| *A. flavus* | 6 | 8 | 8 | 5 |
| *A. niger* | 18 | 20 | 16 | 16 |
| *A. terreus* | 10 | 12 | 12 | 10 |
| *Cladosporium cladosporioides* | 15 | 12 | 10 | 10 |
| *C. herbarum* | 10 | 12 | 10 | 10* |
| *Curvularia lunata* | 25 | 30 | 26 | 21 |
| *Drechslera spicifera* | 20 | 14 | 14 | 14 |
| *Epicoccum nigrum* | 18 | 16 | 16 | 12 |
| *Fusarium chlamydosporum* | 20 | 21 | 18 | 18 |
| *F. oxysporum* | 25 | 17 | 16 | 16 |
| *Nigrospora sphaerica* | 8 | 10 | 12 | 10* |
| *Papulaspora* sp. | 10 | 11 | 9 | 9* |
| *Penicillium chrysogenum* | 16 | 20 | 21 | 18 |
| *P. oxalicum* | 20 | 22 | 18 | 10 |
| *P. purpurogenum* | 0 | 0 | 0 | 0 |
| *Phoma hibernica* | 15 | 15 | 16 | 12 |
| *Trichoderma viride* | 32 | 33 | 30 | 32 |
| *Trichothecium roseum* | 30 | 35 | 32 | 32 |
| Control (radial colony growth in mm on Czapek-Dox + 0.05% yeast extract agar) | 10 | 25 | 35 | 47 |

* Stimulation (%). 2 way analysis of variations showed significant variation among the treatments with different volatile fungal metabolites and incubation periods indicated by the calculated F-value being greater than the tabulated value.
For different volatile fungal metabolites: 104.008 > 2.20 with 22 and 66 degrees of freedom at 1%. For different incubation periods: 7.86 > 4.13 with 3 and 66 degrees of freedom at 1%.

*Results and discussion.* The average colony diameter on a large number of control plates incubated for 96 h was 47 mm ± 5 mm. In other words, 5 mm or 10% inhibition or stimulation in growth could be due to the variability in growth of the pathogen itself. Any variation beyond 10% was thought to be caused by volatile substances excluded from the respective fungus. The results are presented in the table.

Maximum inhibition (more than 20%) in radial colony growth of the pathogen was recorded with *Trichoderma viride* and *Trichothecium roseum* followed by *Aspergillus chevalieri, Curvularia lunata* and *Penicillium chrysogenum. Alternaria humicola, Aspergillus candidus, A. flavus, A. terreus, Cladosporium cladosporiodes, Penicillium oxalicum* and *P. purpurogenum* were found to be least effective or not effective at all. Similarly, Hutchinson and Cowan[5] reported inhibition in growth and sporulation of *Pestalotiopsis rhododendri* in culture due to volatile metabolites of different fungi. These observations of inhibition were perhaps due to active antimicrobial substances comprising HCN (Ward and Thorn[10]), ethylene (Ilag and Curtis[11]), $NH_3$ (Lockwood[7]) and acetaldehyde (Dennis and Webster[3]) present in volatile metabolites of different phylloplane fungi.

On the contrary, in this experiment a slight stimulation of the pathogen was recorded due to the culture volatiles of *Cladosporium herbarum, Nigrospora spaerica* and *Papulaspora* sp., which could be due to a low concentration of $CO_2$, which is stated to be a common fungal growth stimulant (Buston[12]). Similarly Skidmore[8] reported volatile substances from *Aureobasidium* sp. enhancing the growth of *C. herbarum*.

In addition, microscopic investigations showed that volatile metabolites of *Trichoderma viride, Trichothecium roseum* and *Curvularia lunata* caused deformity; vacuolation of the hyphal cytoplasm and stunted mycelial growth. These hyphae were more highly branched than the normal ones. These effects were not permanent, because when replaced on fresh nutrient agar they grew normally. Moreover, observations indicate that volatiles were effective on the youngest portion, i.e. the tip, of a fungal hypha which ceased to grow and started branching just below the tip. The same process of inhibiton and branching occurred with the new side branches arising, and resulted in successively

more resistant hyphae, which might be a possible explanation for the recovery in growth of the pathogen after 24 h. This study suggests that volatile metabolites excluded from some of the nonparastic phylloplane microfungi may effect the growth and development of invading pathogens in an antagonistic manner. However, the present findings are true in vitro, and may not be so on leaf surfaces in natural conditions. Nevertheless, knowledge of such interactions, besides other corroboratory factors[13,14] may, in future, allow manipulation of the phylloplane microflora to maximize their potential for disease control.

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3 C. Dennis and J. Webster, Trans. Br. mycol. Soc. *57*, 41 (1971).
4 S.A. Hutchinson, Trans. Br. mycol. Soc. *57*, 185 (1971).
5 S.A. Hutchinson and M.E. Cowan, Trans. Br. mycol. Soc. *59*, 71 (1972).
6 S.A. Hutchinson, A. Rev. Phytopath. *11*, 223 (1973).
7 J.L. Lockwood, in: Biology and control of soil born plant pathogens, p. 194. Ed. G.W. Bruchl. The American Phytopathological Society, St. Paul 1975.
8 A.M. Skidmore, in: Microbiology of aerial plant surfaces, p. 507. Ed. C.H. Dickinson and T.F. Preece. Academic Press, London 1976.
9 C.H. Dickinson, in: Ecology of leaf surface micro-organisms, p. 129. Ed. T.F. Preece and C.H. Dickinson. Academic Press, London 1971.
10 E.W. B. Ward and G.D. Thorn, Can. J. Bot. *43*, 997 (1965).
11 L. Ilag and R.W. Curtis, Science *159*, 1357 (1968).
12 H.W. Buston, M.O. Moss and D. Tyrell, Trans. Br. mycol. Soc. *49*, 387 (1966).
13 R.K. Upadhyay, D.K. Arora and R.S. Dwivedi, Experientia *36*, 76 (1980).
14 R.K. Upadhyay and D.K. Arora, Experientia *36*, 185 (1980).

## Lack of gravity effect on the speed of amoeboid locomotion in *Naegleria gruberi*[1]

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*Summary.* The speed of amoeboid locomotion was the same for amoebae moving on the top surface and bottom surface of a horizontal perfusion chamber i.e. cell-substrate adhesive forces must be considerably greater than the gravitational force acting on the locomoting amoeba.

In addition to force production[2] in the cytoplasm successful locomotion of amoeboid cells is dependent upon the controlled interactions between the cell surface and a substrate through which traction is mediated. Lacking a mechanism to exert traction, crawling cell locomotion would be ineffective.

In this study we have assessed the effect of gravity on cell traction by measuring the speed at which a soil amoeba *Naegleria gruberi* moved across either of 2 parallel glass coverslips held horizontally within a perfusion chamber[3]. While gravity would be expected to work against the adhesion of cells moving on the upper coverslip, it should have augmented the attachment of amoebae to the lower coverslip. Ambient electrolyte concentration has been shown previously to modulate reversibly not only the cell-substrate gap[3] but also their speed of progression e.g. these protozoons move about 4 times faster in 10 mM KCl than in deionized water[4]. Therefore by varying the ionic milieu in the perfusion chamber we were able to look for any effect of gravity on amoeboid motility over a wide range of locomotory rates.

Freshly harvested cells were introduced into a Prior chamber (W. Prior, Bishops Stortford, Herts., England) the windows of which were round coverslips separated by a lightly greased rubber seal. The medium was changed when necessary as described previously[3]. Amoebae in the chamber were selected at random and their speed of movement recorded over a period of 1 min. The paths taken by individual amoebae were traced by means of a camera lucida and the distances covered in unit time were subsequently determined with a map measuring wheel. In each medium the tracks of at least 40 amoebae were recorded; 20 on the upper coverslip (i.e. inverted) and 20 on the lower coverslip (i.e. noninverted). The experiments were carried out at room temperature (17–18 °C).

The results confirm that the presence of 10 mM KCl (or NaCl) clearly increased the speed of locomotion over that attained in deionized $H_2O$. Furthermore a similar increase was not observed when an osmotically equivalent (20 mM) solution of the non-electrolyte sucrose was used. However one could not show any differences between inverted and noninverted cells with regard to their speed of movement in specified media. It would therefore appear that the cell-substrate adhesive forces between *Naegleria* amoebae and glass were much greater than the force of gravity acting on the mass of the cell.

Gingell et al.[5] have studied the adhesion of fixed red blood cells to an inverted oil/$H_2O$ interface. By reducing the ionic strength of the suspending solution, the cell-interface gap was increased[6]. When the strength of the medium was reduced to 0.3 mM NaCl about half the cells fell off. This could be viewed as a balance point between the gravitational force acting on the red cell and the adhesive force acting between the cell and the oil-water interface. The final adhesive force was considered to be produced by a balance between the long range forces of attraction (probably Van der Waals forces) and repulsive electrostatic forces set up between 2 surfaces of like charge (i.e. negatively charged cell and negatively charged interface). This situation obviously did not apply to these experiments with *Naegleria* where the ionic strength of medium can be reduced to the equivalent of 0.01 mM NaCl (= deionized water used in this study) without the cells falling off. The nature and extent of the cell-substrate interaction in *Naegleria* amoebae will be very different because of the presence of punctate focal contacts and an extensive area of 'associated contact'[3]. Although raising the ionic strength of the medium increased speed of movement there was not a similarly significant increase in the number of focal contacts produced[4]. Anyway the effect of focal contacts will be