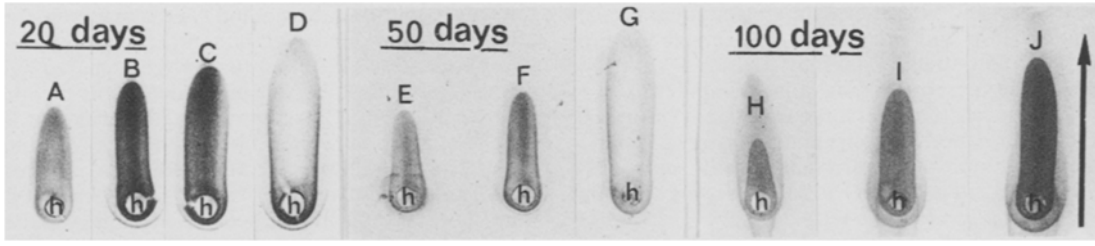


Figure 3. Rocket immunoelectrophoresis of *A. obtectus* haemolymph by Laurell's technique, h, haemolymph. A, E, H: virgin females without mature oocytes in the absence of bean seeds fed with honeyed water; B, F, I: virgin females with mature oocytes in absence of bean seeds fed with honeyed water; C: virgin females with mature oocytes in absence of bean seeds fed with honeyed water and pollen. D. G. J: virgin females with mature oocytes in presence of bean seeds fed with honeyed water and pollen.

d) Among females fed on pollen, rocket heights were similar to those found for controls (adults without bean seeds). High ovarian production, especially at 100 days, was not accompanied by a decrease in vitellogenin content. Therefore, vitellogenin content can only be maintained via synthesis from pollen constituents.

*Discussion.* In reproductively inactive females less than 100 days old, the presence of pollen in the adult diet was not an adequate stimulus to induce oocyte maturation, in marked contrast with what has been observed in *Bruchus pisorum*[4]. Vitellogenin (when synthesized) was maintained at a very low level. However, we emphasize that the pollen supplied to the bruchids in our experiments was not from the flowers of the host plant. On the other hand, for females aged 100 days or more, a diet containing this pollen allowed production of oocytes which accumulated in the lateral oviducts. In the absence of host-plant seeds, these oocytes were not laid.

Thanks to the reproductive quiescence of a fraction of the female population, *Acanthoscelides obtectus* would be able to wait for the maturation of *Phaseolus vulgaris* pods and seeds, neither producing nor releasing oocytes. Through this adaptation, eggs with small survival chances would probably not be laid[2,3]. If feeding was the stimulus inducing vitellogenesis in the early days of imaginal life, as in some Diptera and Lepidoptera[15], the insect's reproductive cycle might well be out of phase with that of the host plant.

In reproductively active females, provision of a protein diet increased both the haemolymph's protein content and ovarian production, as observed in *Musca domestica*[16] and *Oxya japonica*[17]. This food supply helped to maintain the vitellogenin content in the haemolymph even when oocytes were produced and then released in the presence of *Phaseolus vulgaris* seeds.

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## Sporostasis between phylloplane microfungi and a foliar pathogen

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*Summary.* Interactions between germinating spores of phylloplane microfungi and *Pestalotiopsis funerea* Desm., a leaf spot pathogen of *Eucalyptus globulus* Labill., were studied in vitro. Findings suggest that sporostasis imposed on *P. funerea* by germinating spores of other nonparasitic microfungi was due to nutrient impoverishment or mycotoxins present in spore exudates.

Much work has been done on staling growth products leading to mycostasis between pathogenic and saprophytic fungi in a variety of microecological niches[3,4]. In our[5,6] previous studies on leaf inhabiting microfungi, we reported on the antimicrobial activity of staling growth substances of phylloplane fungi against *P. funerea* due to the presence of antibiotics as a major factor besides other minor causes like alteration in pH and nutrient impoverishment. Recently, Blakeman and Brodie[7] reviewed the literature and suggested that epiphytic bacteria inhibit the growth of foliar pathogens by creating a nutrient shortage during spore germination and successive disease development, analogous to the theory propounded for soil fungistasis by Ko and Lockwood[8]. However, it is evident from the literature[9,10] that nonparasitic fungi already growing on leaf surfaces or applied artificially were strongly antagonistic against developing pathogens, leading to biological control. Microscopic observations[11] showed that most of the microfungi remained on the leaf surfaces in the form of spores except for a few mycelial forms. Therefore, it is expected

that spores of parasitic as well as nonparasitic fungi present in an infection drop will interact by their metabolic produce of germination affecting thereby the future course of pathogenesis. The present investigation was undertaken to deal with both aspects; nutrient impoverishment caused by, and mycotoxins produced by germinating spores of phylloplane microfungi on spore germination and germ tube extension of *Pestalotiopsis funerea* Desm. causing leaf spot of *Eucalyptus globulus* Labill. in vitro.

*Materials and methods.* Monosporic cultures of various phylloplane fungi including *P. funerea* isolated from the leaf of *E. globulus* by different cultural techniques described by Dickinson[12], were maintained on Czapek-Dox + 0.05% yeast extract agar medium for further study. Spores were harvested from 8-day-old cultures by flooding Petri dishes with sterile distilled water. They were washed twice with deionized water by low speed centrifugation (800 rpm) before their germination rate was tested. Spore suspensions of 50–60 spores/0.01 ml were prepared for each fungal strain investigated. Equal volumes of the above spore suspension of *P. funerea* and an antagonist (a phylloplane microfungus) was mixed and placed in a cavity slide to assess the percentage of spore germination and rate of germ tube extension at different incubation times. Control data were recorded using spores of a single fungus only. Determination of total carbohydrate by the colour reaction given by the anthrone reagent[13] and total amino acids by a ninhydrin colorimetric method[14] were made on glass slides after filtering to remove germinating fungal spores.

Spore exudates of different fungi were prepared by keeping spores ($16 \times 10^6$ spores/ml) in sterile double distilled water for 48 h at 24 ± 1 °C in alternate 12 h periods of light and darkness. They were then filtered through Whatman filter paper No. 44 followed by Seitz filtration. In the case of *Papulospora* sp. a mycelial extract (ME) was prepared by mincing mycelium in distilled water, boiling and filtering. It was partially purified by adsorption to activated charcoal, and then evaporated to reduce the volume. Effects of these exudates or ME on spores germination of *P. funerea* were studied as above.

*Results and discussion. Aspergillus flavus, Fusarium oxysporum* and *Penicillium oxalicum* were found to be most effective, causing more than 60% inhibition of spore germination and 75% of germ tube extension of *P. funerea.* It is evident from table 1 that 80% of the spores of *P. funerea* germinated well without any external supply of nutrients. Total carbohydrate and amino acid analyses of water containing germinating spores of *P. funerea* showed that they reached a maximum after 6 h of incubation followed by a decrease. Table 2 depicts continuous replacement of the solution containing germinating spores of *P. funerea* by fresh sterile double distilled water using micropipettes after 3, 6, 12 and 24 h of incubation reduced the percentage germination and germ tube extension considerably. It

Table 1. Spore germination, germ tube extension of *Pestalotiopsis funerea* alone or mixed with an antagonist, along with analyses of water droplets containing germinating spores for carbohydrate and amino acids on glass slides

| Treatment* | Incubation period in h | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | | | | 6 | | | | 12 | | | | 24 | | | | | |
| | g | gt | C | A | g | gt | C | A | g | gt | C | A | g ± SD | | gt ± SD | | C ± SD | A ± SD |
| *Pestalotiopsis funera* (P) | 10 | 22 | 10 | 0.09 | 35 | 90 | 20 | 0.38 | 75 | 210 | 15 | 0.12 | 80 | 1.6 | 245 | 8.5 | 16 0.16 | 0.05 0.008 |
| *Alternaria alternata* | – | – | 8 | 0.01 | 20 | – | 15 | 0.14 | 43 | – | 18 | 0.16 | 60 | 2.2 | – | – | 20 0.21 | 0.05 0.016 |
| P × *A. alternata* | 10 | 20 | 15 | 0.12 | 33 | 40 | 30 | 0.43 | 60 | 100 | 28 | 0.21 | 65 | 2.0 | 140 | 4.1 | 28 0.29 | 0.10 0.020 |
| *Aspergillus flavus* | 0 | – | 6 | 0.05 | 40 | – | 10 | 0.01 | 58 | – | 15 | 0.10 | 69 | 2.6 | – | – | 12 0.21 | 0.08 0.008 |
| P × *A. flavus* | 0 | 0 | 20 | 0.16 | 22 | 23 | 34 | 0.41 | 30 | 42 | 28 | 0.28 | 35 | 0.8 | 48 | 8.5 | 32 0.43 | 0.26 0.02 |
| *Aureobasidium pullulans* | – | – | 10 | 0.11 | – | – | 16 | 0.23 | – | – | 12 | 0.18 | – | – | – | – | 8 0.25 | 0.11 0.01 |
| P × *A. pullulans* | 5 | 12 | 20 | 0.18 | 20 | 49 | 24 | 0.41 | 46 | 57 | 22 | 0.20 | 50 | 1.5 | 65 | 2.1 | 20 0.31 | 0.15 0.02 |
| *Cladosporium cladosporioides* | 10 | – | 10 | 0.09 | 45 | – | 12 | 0.18 | 85 | – | 15 | 0.10 | 95 | 2.8 | – | – | 11 0.21 | 0.01 0.0 |
| P × *C. cladospoioides* | 8 | 18 | 14 | 0.10 | 28 | 60 | 30 | 0.25 | 50 | 90 | 20 | 0.18 | 60 | 3.7 | 120 | 7.2 | 18 0.16 | 0.09 0.008 |
| *C. herbarum* | 8 | – | 12 | 0.09 | 40 | – | 18 | 0.23 | 75 | – | 20 | 0.18 | 100 | 0.0 | – | – | 16 0.29 | 0.09 0.002 |
| P × *C. herbarum* | 10 | 24 | 20 | 0.21 | 30 | 70 | 36 | 0.38 | 60 | 110 | 28 | 0.20 | 65 | 3.5 | 148 | 8.4 | 20 0.21 | 0.10 0.014 |
| *Curvularia lunata* | – | – | 10 | 0.05 | 15 | – | 15 | 0.13 | 48 | – | 16 | 0.19 | 55 | 2.1 | – | – | 18 0.37 | 0.10 0.02 |
| P × *C. lunata* | 10 | 20 | 18 | 0.12 | 36 | 43 | 26 | 0.42 | 50 | 80 | 25 | 0.20 | 60 | 2.4 | 110 | 0.9 | 22 0.37 | 0.10 0.0 |
| *Epicoccum nigrum* | – | – | – | – | 15 | – | 15 | 0.28 | 25 | – | 16 | 0.21 | 35 | 2.6 | – | – | 18 0.47 | 0.11 0.01 |
| P × *E. nigrum* | 10 | 15 | – | – | 30 | 60 | 30 | 0.38 | 63 | 180 | 32 | 0.20 | 85 | 2.2 | 260 | 12.3 | 24 0.43 | 0.15 0.02 |
| *Fusarium oxysporum* | 5 | – | 6 | 0.01 | 20 | – | 8 | 0.09 | 53 | – | 10 | 0.10 | 75 | 2.6 | – | – | 10 0.16 | 0.01 0.0 |
| P × *F. oxysporum* | 0 | 0 | 14 | 0.10 | 20 | 45 | 34 | 0.40 | 28 | 50 | 30 | 0.30 | 30 | 1.6 | 50 | 6.6 | 32 0.49 | 0.28 0.01 |
| *Papulospora* sp. | – | – | 12 | 0.21 | – | – | 10 | 0.26 | – | – | 12 | 0.21 | – | – | – | – | 8 0.43 | 0.20 0.02 |
| P. × *Papulospora* sp. | 20 | 35 | 24 | 0.21 | 60 | 180 | 28 | 0.50 | 100 | 456 | 26 | 0.26 | 100 | 0.0 | M | – | 22 0.37 | 0.18 0.02 |
| *Penicillium oxalicum* | 15 | – | 6 | 0.01 | 38 | – | 8 | 0.09 | 60 | – | 10 | 0.10 | 80 | 1.6 | – | – | 10 0.21 | 0.01 0.0 |
| P × *P. oxalicum* | 0 | 0 | 14 | 0.10 | 15 | 45 | 25 | 0.40 | 25 | 40 | 30 | 0.30 | 28 | 2.6 | 40 | 4.3 | 32 0.35 | 0.28 0.01 |
| *Phoma hibernica* | 0 | 0 | – | – | 10 | – | 8 | 0.10 | 30 | – | 12 | 0.18 | 45 | 1.8 | – | – | 14 0.21 | 0.10 0.001 |
| P × *P. hibernica* | 5 | 13 | – | – | 21 | 57 | 24 | 0.35 | 53 | 77 | 28 | 0.25 | 55 | 2.4 | 80 | 2.1 | 20 0.35 | 0.26 0.01 |

g, Percent spore germination; gt, germ tube extension (μm); C, total carbohydrate (μg/ml); A, total amino acids (μM/ml); M, changed into mycelial form. * Population of spores in cavity slide was 50–60 spores/0.01 ml of water.

Table 2. Percent spore germination (g) and germ tube extension (gt, μm) of *P. funerea* in fresh replaced water at different intervals of time

| Treatment | Incubation time in h | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | | 6 | | 12 | | 24 | | | |
| | g | gt | g | gt | g | gt | g ± SD | | gt ± SD | |
| Water control | 10 | 20 | 35 | 90 | 75 | 210 | 80 | 1.6 | 245 | 8.5 |
| Fresh water after replacement | 8 | 20 | 15 | 20 | 25 | 30 | 30 | 1.2 | 40 | 5.4 |

remains to be determined whether this reduction results from a removal of nutrients from the incubation medium by the replacement technique or from an accelerated leakage of spore material induced by this technique.

Since cogermination of *P. funerea* with the 3 fungi mentioned above does not reduce the carbohydrate or amino acid content of the bathing solution (table 3), the reduction of the germination rate in *P. funerea* could possibly be due to the presence of germination inhibitors. Observations of a similar nature are summarized in the review paper by Allen[15]. pH changes do not seem to be of significance as the pH stays within the range of 6.5–7.5 which completely overlaps with the optimal range for germination.

Mycelial extract (ME) of *Papulospora* sp. stimulated spore germinating and germ tube extension of *P. funerea* significantly. This was perhaps due to the presence of some growth promoting substances in ME; a similar effect was observed previously by us[6,7], in studies of induced growth and colonization of *P. funerea* in metabolites of *Papulospora* sp. *Nigrospora sphaerica* and *Epicoccum* nigrum were found to stimulate spore germination and germ tube extension to some extent, possibly by increasing the nutrient level in the external fluid (table 1) containing the spore mixture.

These findings suggest that nonparasitic phylloplane fungi may antagonize foliar pathogens by creating nutrient shortage or producing inhibitory substances on leaf surfaces in an ecological niche. It should be remembered, however, that these experiments apply to an in vitro situation and may not necessarily hold true in vivo.

Table 3. Effect of spore exudates of different phylloplane fungi on the spore germination and germ tube extension of *P. funerea*

| Name of fungi | Percent spore germination (g) and germ tube extension (gt, µm) at different intervals of time (h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 6 | | 12 | | 24 | | | |
| | g | gt | g | gt | g ± SD | | gt ± SD | |
| Water control | 30 | 90 | 80 | 210 | 85 | 0.8 | 280 | 10.8 |
| *Alternaria alternata* | 23 | 40 | 35 | 80 | 46 | 3.7 | 120 | 5.4 |
| *Aspergillus flavus* | 10 | 10 | 15 | 20 | 18 | 2.9 | 33 | 1.4 |
| *Aureobasidium pullulans* | 20 | 46 | 65 | 110 | 70 | 2.6 | 160 | 5.1 |
| *Cladosporium cladosporioides* | 20 | 65 | 68 | 115 | 90 | 2.1 | 200 | 3.2 |
| *C. herbarum* | 25 | 80 | 75 | 180 | 100 | 0.0 | 260 | 5.3 |
| *Curvularia lunata* | 20 | 30 | 35 | 38 | 40 | 2.9 | 43 | 2.6 |
| *Epicoccum nigrum* | 35 | 60 | 56 | 115 | 90 | 1.7 | 300 | 4.8 |
| *Fusarium oxysporum* | 0 | 0 | 26 | 10 | 30 | 2.3 | 23 | 2.1 |
| *Nigrospora sphaerica* | 25 | 40 | 60 | 160 | 90 | 2.0 | 296 | 7.7 |
| *Papulospora* sp. (ME) | 60 | 120 | 100 | 280 | 100 | 0.0 | – | |
| *Penicillium oxalicum* | 0 | 0 | 20 | 10 | 28 | 1.6 | 20 | 2.1 |
| *Phoma hibernica* | 0 | 0 | 33 | 30 | 45 | 1.5 | 58 | 3.6 |

ME, Mycelial extract.

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## Electrical impedance of daminozide- and calcium-treated McIntosh apples

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*Summary.* Daminozide [butanedioc acid mono-(2-2-dimethylhydrazide)] applied in the field reduced the electrical impedance of McIntosh apple fruit at harvest and after storage. Vacuum infiltration with calcium chloride ($CaCl_2$) increased the impedance whether fruit were treated with daminozide or not.

Bioelectric tests have been used to evaluate many characteristics of plants or plant organs such as frost hardiness in woody ornamentals and trees[2], virus infection[3], and fruit senescence[4]. Impedance is one bioelectric test often applied because of the ease of measurement and its apparent correlation with pathological and physiological changes. Electrical impedance of peaches has been shown by Weaver and Jackson[5] to increase until fruit reach the ripe stage after which it declines. Golovkin and Tsvetkov[6] used impedance as an indication of the change in the permeability of apple membranes at reduced temperature. Greenham[7] demonstrated that the impedance of bruised apples was lower than those not bruised, and Sacher[4] referred to the use of impedance as a measurement of senescence and, indirectly, cell permeability.

Looney[8] suggested that daminozide could influence respiration through maintenance of membrane integrity in apples. Unduragga and Ryugo[9] found that permeability of almond seedlings was increased by daminozide treatment and proposed this permeability change was effective in