to electrophoresis is not necessary. The method has a high sensitivity and is applicable to studies dealing with µg quantities of tissues.

1 Acknowledgments. The financial support by the Swiss National Science Foundation and the George and Antoine Claraz-Schenkung is gratefully acknowledged. I thank Prof. Max Birnstiel for providing me with the sea urchin histone mRNA and Prof. Martin Billeter for providing me with the rabbit globin mRNA as RNA markers.

2 P.S. Chen, Biochemical Aspects of Insect Development. Karger, Basel 1971.
3 P.S. Chen, in: Biochemistry of Insects, p. 145. Ed. M. Rockstein. Academic Press, New York 1978.
4 E. von Wyl and P.S. Chen, Rev. suisse Zool. *81*, 655 (1974).
5 D.Z. Staynov, J.C. Pinder and W.B. Gratzer, Nature, New Biol. *235*, 108 (1972).
6 H.K. Mitchell, P.S. Chen, L.S. Lipps and G. Moller, Insect Biochem. *8*, 29 (1978).
7 W.M. Bonner and R.A. Laskey, Eur. J. Biochem. *46*, 83 (1974).
8 P.S. Chen, in: Invertebrate Tissue Culture, Ed. E. Kurstak and K. Maramorosch. Academic Press, New York (in press).
9 D.B. Roberts, J. Wolfe and M.E. Akam, J. Insect Physiol. *23*, 871 (1977).
10 G. Korge, Devl Biol. *58*, 339 (1977).
11 S.K. Beckendorf and F.C. Kafatos, Cell *9*, 365 (1976).

Role of fungal staling growth products in inter-specific competition among phylloplane fungi

R.K. Upadhyay[1,2] and D.K. Arora

Laboratory of Mycology and Plant Pathology, Department of Botany, Banaras Hindu University, Varanasi-221005 (India),
28 February 1979

Summary. The effect of fungal staling growth products on leaf-inhabiting microfungi, with special reference to a leaf spot pathogen *Pestalotiopsis funerea* Desm. of *Eucalyptus globulus* Labill. was studied. Results depict that antibiotics produced by competing microfungi caused the phenomenon of mycostasis on the leaf surfaces.

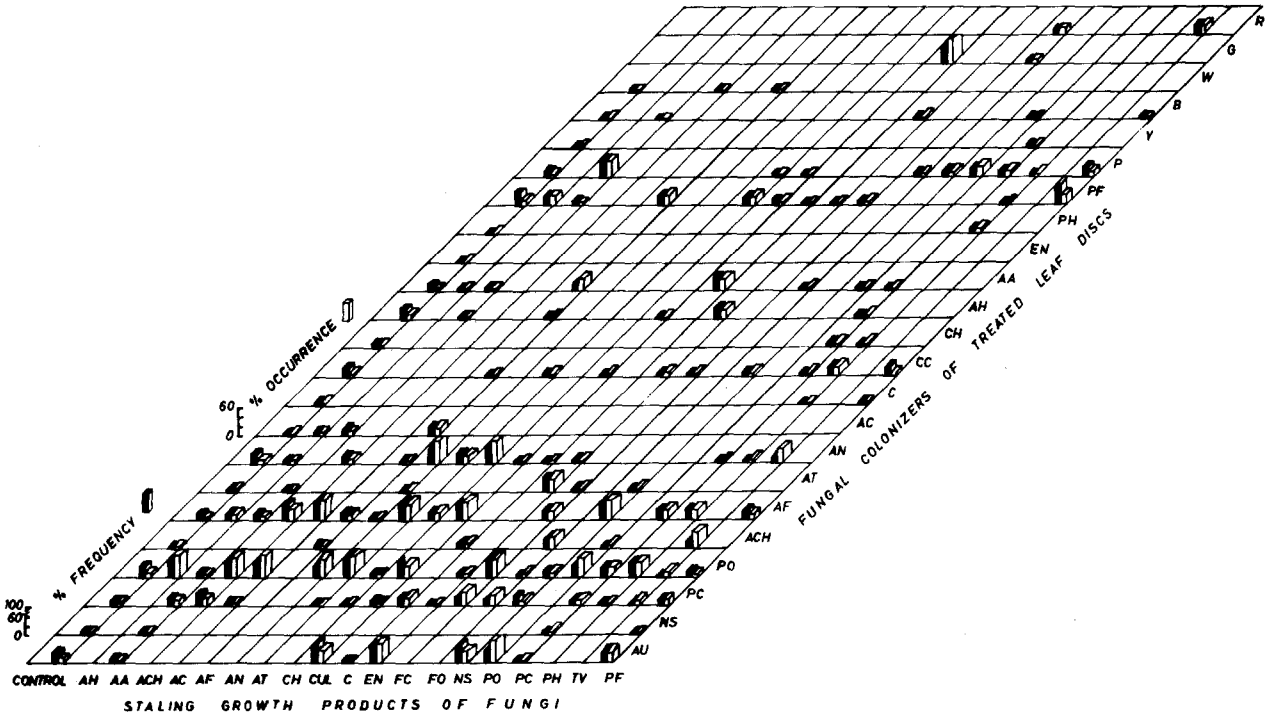
Leaf-inhabiting microfungi may inhibit the development of invading pathogens by creating a nutrient shortage or by producing inhibitory metabolic substances in an antagonistic manner[3-5]. As yet, few studies have been undertaken on staling growth products leading to the phenomenon of mycostasis among leaf-inhabiting microfungi. In the present investigation, staling growth products of various leaf-inhabiting microfungi of *Eucalyptus globulus* Labill. were studied for their effect on microfungal colonization of leaf discs with special reference to *Pestalotiopsis funerea* Desm., a leaf spot pathogen.

Materials and methods. Leaf-inhabiting microfungi of *E. globulus* were isolated by different techniques described by Dickinson[6], and maintained in pure cultures on Czapek-Dox+0.05% yeast extract agar. For the preparation of staling growth products of these fungi, 250 ml Erlenmeyer flasks containing 100 ml liquid nutrient Czapek-Dox+0.05% yeast extract were taken, and each was inoculated with 2 6-mm mycelial discs of the respective fungus. The flasks were incubated for 120 h at 25±1 °C on a reciprocating shaker (100 shakes per min with a 10-cm coverage) and cultures were filtered through Whatman filter paper No. 42 and finally through a Seitz filter. The pH of all the staling growth products was adjusted to 5.8 before treatment. The effect of staling growth products with reference to their antibiotic action on leaf colonization was studied by an immersion method. 40 leaf discs (10-mm size) were soaked in 30 ml of a particular fungal culture filtrate for 48 h and then blotted dry in folds of sterile filter papers. 4 such treated discs were placed on solidified Czapek-Dox+0.05% yeast extract agar in each plate. 10 replicates for each treatment were prepared and a suitable control was maintained by inoculating leaf discs treated only with liquid Czapek-Dox+0.05% yeast extract. They were incubated at 25±1 °C and examined after 7 and 14 days for developing fungal colonies.

Results and discussion. When leaf discs were treated with different fungal staling growth products, a significant decrease in the number of fungal species was observed. 21 species (17 Deuteromycetes and 4 sterile mycelia) appeared in the control, whereas there were not more than 12 species in any of the treated groups (figure).

Alternaria alternata, A. humicola, Aspergillus flavus, A. niger, Aureobasidium pullulans, Cladosporium cladosporioides, Penicillium oxalicum and *Pestalotiopsis funerea* colonized the untreated (control) leaf discs frequently; however, only *A. flavus, A. niger, Penicillium chrysogenum* and *P. oxalicum* were frequent on the treated leaf discs, thereby showing their good tolerance capacity for the staling growth products of other fungi. *Aspergillus candidus, Cephalosporium roseum, Cladosporium herbarum, Nigrospora sphaerica* and *Phoma hibernica* seemed to be highly susceptible, since they occurred rarely on the treated discs. Overall, staling growth products of *A. candidus, A. chevalieri, A. terreus, C. roseum, Penicillium* spp. and *Trichoderma viride* were highly toxic to the phylloplane fungi colonizing treated leaf discs, whereas *A. alternata, Curvularia lunata, Epicoccum nigrum, P. hibernica* and *P. funerea* were less effective.

P. funerea was found to be intolerant of (most susceptible to) the staling growth products of *A. chevalieri, A. candidus, A. terreus, Fusarium oxysporum, F. chlamydosporum, N. sphaerica, P. oxalicum, P. chrysogenum* and *T. viride,* as they did not allow it to appear on any of the treated discs. *P. funerea* was not very susceptible to the staling growth products of *A. alternata, C. lunata, C. roseum, F. chlamydosporum* and *P. hibernica* as it colonized treated discs to some extent. It was found to be most tolerant to the liquid metabolites of *A. humicola, A. flavus* and *C. herbarum,* in which its percentage occurrence was more than in the control. Likewise, *A. humicola, A. flavus, A. niger, A. pullulans, Papulospora* sp, *P. chrysogenum* and *P. oxalicum* exhibited an increase in percentage occurrence on the treated discs with certain metabolites. Speculation about the reason for this observation is that these fungi possessed a better capacity of tolerance to the staling growth substances as compared with the others. Furthermore, some growth substances present in the metabolites[7] might be a possible



Effect of fungal staling growth products on percentage occurrence and frequency of leaf inhabiting microfungi of *Eucalyptus globulus* Labill. estimated by the immersion method.
AH = *Alternaria humicola*, AA = *A. alternata*, AC = *Aspergillus candidus*, ACH = *A. chevalieri*, AF = *A. flavus*, AN = *A. niger*, AT = *A. terreus*, AU = *Aureobasidium pullulans*, C = *Cephalosporium roseum*, CC = *Cladosporium cladosporioides*, CH = *C. herbarum*, CUL = *Curvularia lunata*, EN = *Epicoccum nigrum*, FC = *Fusarium chlamydosporum*, FO = *F. oxysporum*, NS = *Nigrospora sphaerica*, P = *Papulospora* sp, PC = *Penicillium chrysogenum*, PO = *P. oxalicum*, PF = *Pestalotiopsis funerea*, PH = *Phoma hibernica*, TV = *Trichoderma viride*, B = brown sterile fungus, G = green sterile fungus, R = red sterile fungus, W = white sterile fungus, Y = yellow sterile fungus.

factor responsible for their stimulatory action on colonization.

Some fungal species, namely *A. chevalieri*, *A. terreus*, *C. roseum*, and *E. nigrum*, did not appear on leaf discs treated with their own staling substances. Perusal of the figure shows that the percentage colonization and frequency of these fungal species is relatively very low in the control, revealing their lower inoculum potential[8], resulting in a decrease in colonization capacity. Furthermore, these fungi grew fast and attained earlier maximal growth in the liquid culture medium during metabolite preparation, which caused autolysis and thereafter changes[7,9] leading to inhibition of their own growth.

The inability of any fungus to colonize the treated discs might be attributed to the direct effect of fungal staling growth substances on spore germination and hyphal extension. It may be presumed that staling substances either formed a metabolite coating[10] around the reproductive bodies or vegetative mycelium, or were absorbed within them and caused a hypersensitive action, which ended their growth and colonization. Fungal forms with a capacity to annul the inhibitory action of staling substances by adopting a different course of resistance[11] would colonize better. Good tolerance capacity as a main factor for successful colonization was emphasized by Arora and Upadhyay[12], and Park[13].

The possible factors present in fungal staling growth substances causing inhibition are antibiotics, pH alteration and nutrient depletion[3–5]. The latter 2 factors (pH and nutrients) might be neglected, as the pH of all the metabolites was maintained at 5.8 before immersion of leaf discs, and there after discs were placed on nutrient-rich/Czapek-Dox × 0.05% yeast extract agar for colonization. Thus the presence of antibiotics, out of the 3 possibilities, might be the main inhibiting factor in the staling growth substances. Our study provides an understanding of the potential competitors among the leaf-inhabiting microfungi creating adverse conditions for a developing foliar pathogen in a particular ecological niche. This antibiotics theory might be put forward as an alternative to the view of nutrient impoverishment for the mycostasis phenomenon on leaf surfaces.

1 Acknowledgments. I would like to express my thanks to Professor R.S. Dwivedi for his encouragement and to CSIR (Indian Govt.) for financial assistance.
2 Address for reprint requests: Dr R.K. Upadhyay, Department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India.
3 N.J. Fokkema, in: Microbiology of aerial plant surfaces, p. 486. Academic Press, London 1976.
4 A.M. Skidmore, in: Microbiology of aerial plant surfaces, p. 507. Academic Press, London 1976.
5 R.K. Upadhyay and R.S. Dwivedi, Proc. Indian natl Sci. Acad. *43B*, 33 (1977).
6 C.H. Dickinson, in: Ecology of leaf surface micro-organisms, p. 129. Academic Press, London 1971.
7 W.B. Turner, Fungal metabolites. Academic Press, London 1971.
8 S.D. Garrett, in: Biology of root-infecting fungi, p. 132. Cambridge University Press, London 1956.
9 D. Park, Trans. Br. mycol. Soc. *44*, 377 (1961).
10 A.N. Shukla, D.K. Arora and R.S. Dwivedi, Soil Biol. Biochem. *9*, 217 (1977).
11 J. Dekker, A. Rev. Phytopath. *14*, 405 (1976).
12 D.K. Arora and R.K. Upadhyay, Plant Soil *49*, 685 (1978).
13 D. Park, in: The ecology of soil fungi, p. 148. Liverpool University Press, 1960.