Plant and Soil 106, 285–288 (1988)

PLSO 6234

Effect of metabolites of *Aspergillus niger* and *Trichoderma viride* on development and structure of radicle of cocoa (*Theobroma cacao*) seedlings

G.T. ODAMTTEN and G.C. CLERK[1]
Department of Botany, University of Ghana, P.O. Box 55, Legon/Accra, Ghana. [1]*Present address: Department of Botany, University of Port Harcourt, Port Harcourt, Nigeria*

Received 3 January 1985. Revised September 1987

Key words: *Aspergillus niger*, cocoa, fungi, radicles, *Theobroma cacao*,*Trichoderma viride*

Abstract

Both the volume and concentration of filtrate of *Aspergillus niger* and *Trichoderma viride* influenced the development of the radicles of cocoa seedlings. Radicles placed in Petri dishes containing filter paper moistened with 20 and 30 ml of undiluted filtrate and filtrate dilutions of 1:1 and 1:2 failed to develop lateral roots and eventually died.

The culture filtrate of *A. niger* was more repressive. Radicles in the same volume of filtrate (20 and 30 ml) of higher dilutions (1:5 and 1:10) developed lateral roots and survived. Radicles placed in less volume ($\leqslant$ 10 ml) survived and produced lateral roots irrespective of concentration of filtrates. Development of the radicles and roots in the control was consistently better ($P = 0.05$) than in filtrate solutions of either *A. niger* or *T. viride*.

The hypocotyls of seedlings under the influence of metabolites of *A niger* showed greater cambial activity and formed xylem vessels and tracheids with larger lumina.

Introduction

Metabolites of non-pathogenic fungi may have adverse effects on plants. Important observations include suppression of seed germination (Leelavathy, 1969; Narain and Prakash, 1968) and malformation and retardation of growth of seedlings (Bowen and Rovira, 1961; Curtis, 1958a, b). In many of these studies, the fungi involved included *Aspergillus niger*, Van Tieghem and *Trichoderma viride* Pers ex Fr.

The metabolites of *A. niger* and *T. viride* reduced sporangial size and inhibited vegetative growth and sporangial formation as well as direct and indirect sporangium germination and zoospore motility of *Phytophthora palmivora* (Butl) Butl (Odamtten and Clerk, 1980; 1985). *P. palmivora* causes black pod disease of cocoa in many parts of the world.

The two soil fungi, *A. niger* and *T. viride* have been suggested as possible biological control agents for *P. palmivora* in soil (Odamtten and Clerk, 1980; 1985). The most important question to ask is whether *A. niger* and *T. viride* are completely harmless to cocoa plant to allow their being employed as biological control agents against *P. palmivora*.

The aim of these studies reported here was to examine the effects of the culture filtrate of *A. niger* and *T. viride* on the development of radicles and the growth of cocoa seedlings.

Materials and methods

Culture filtrates of *A. niger* and *T. viride* were obtained by culturing *A. niger* in V-8 Broth (at pH 2.5) for 4 days at 35°C and *T. viride* in V-8 Broth (at pH 4.2) for 4 days at 30°C.

Effect of cultural filtrates on the development of radicles of cocoa seedlings

The testa of the beans were removed and the beans surface-sterilized with 0.1% mercuric

Table 1. Effect of filtrate volume and concentration of *A. niger* and *T. viride* on radicle development at 27°C after 3 days incubation

Source and dilution of filtrate	pH of medium	Mean length of radicle (mm) in indicated volume of filtrate				Mean dry weight (mg) of radicles in indicated volume of filtrate			
		5.0 ml	10.0 ml	20.0 ml	30.0 ml	5.0 ml	10.0 ml	20.0 ml	30.0 ml
A. niger									
Undiluted	2.6	20.0	19.8	10.6[a]	16.7[a]	305	450	410	400
1:1	2.5	38.0	24.8	17.4[a]	20.2[a]	313	432	430	470
1:2	2.4	42.8	39.2	30.9[a]	30.2[a]	310	430	430	475
1:5	2.3	51.2	41.2	37.6	30.7	312	432	425	470
1:10	2.3	51.6	52.2	46.1	31.2	313	435	430	470
T. viride									
Undiluted	6.4	39.1	23.8	24.8[a]	23.5[a]	312	460	450	420
1:1	6.3	54.7	26.8	26.8[a]	24.7[a]	309	470	460	475
1:2	6.2	66.3	46.2	31.5[a]	31.3[a]	320	470	463	489
1:5	6.0	65.2	50.1	41.2	31.8	320	465	460	489
1:10	5.9	60.2	54.8	48.5	32.3	319	470	470	485
Distilled water (control)	6.9	83.9	84.3	83.7	83.4	398	493	495	510

[a] Radicles died without producing lateral roots.

chloride. The beans were pre-germinated for 4 days in sterile vermiculite and were removed when the radicles were about 10 mm long. Previously germinated cocoa beans were placed on filter paper on petri plates moistened with distilled water (control) or with 2.5, 5.0, 10.0, 20.0 or 30.0 ml of culture filtrate of either *A. niger* or *T. viride*. For each volume of culture filtrate employed, the filtrate was used either undiluted or diluted 1:1, 1:2, 1:5 and 1:10. There were fifteen replicates per treatment. To bring the radicles in contact with the moist filter paper, the plumular end was raised with 6 mm thick glass rod. The setup was then exposed to the normal tropical day/night light regime at 27°C for 3 days.

Anatomical studies

The structure of hypocotyls of seedlings growing

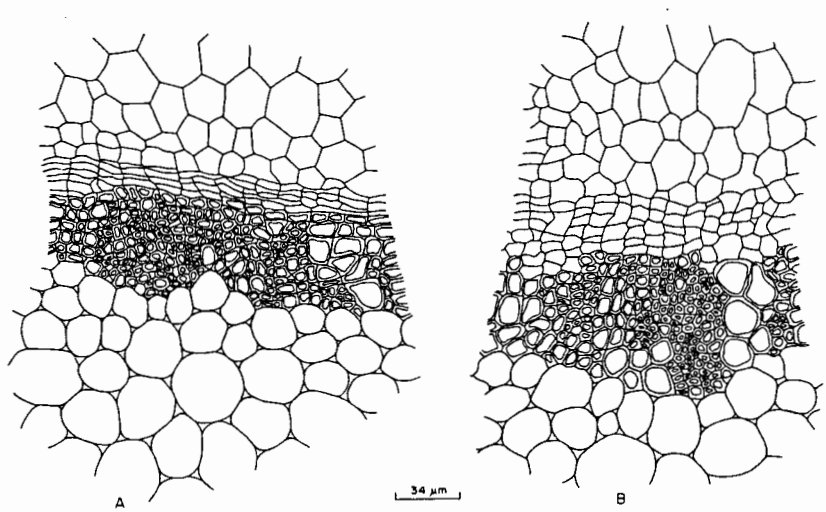

Fig. 1. Camera lucida drawing of T.S. of radicle of 7 day-old cocoa seedling in A. Filtrate-free medium (control) B. Treated with undiluted filtrates of *A. niger*

case of radicles placed in more dilute filtrates, growth of seedlings was markedly retarded. Metabolites of *A. niger* and *T. viride* similarly retarded growth of seedlings in potted cocoa plants (Odamtten and Clerk, 1985). Postlethwait and Curtis (1959) suggested that *A. niger* probably produces IAA-like substances which when applied to stems and leaf petioles of *Phaseolus vulgaris* var 'Black Valentine' consequently stimulated cell division and growth. The stems and leaf petioles exhibited severe curvatures, increased diameter, reduced length and formed irregular protrusions. It is likely that *A. niger* produced similar substances in this study. Radicles treated with filtrate of *A. niger* showed greater accumulated cambial derivatives and the xylem vessels and tracheids were larger in diameter than those produced in control plants (Figs 1 and 2).

Toxic metabolites of *A. niger* and *T. viride* have been shown to be inhibitory in various stages of the life cycle of *P. palmivora* (Odamtten and Clerk, 1985). *A. niger* was still potent even at 1:200 dilutions; a concentration totally harmless to cocoa seedlings. The practical conclusion from our findings is that it would be possible to use particularly *A. niger* as biological control agent against *P. palmivora* in soil. What remains to be worked out is the amount of inoculum which could judiciously be added to soil to produce sufficiently low concentrations of the metabolite as to be harmless to the cocoa plant but potent enough to destroy the pathogen.

Acknowledgement

We thank the Ghana Cocoa Growing Research Association Limited, Birmingham, England for their financial support.

References

Bowen G D and Rovira A D 1961 Soil Sci. 15, 166–186
Curtis R W 1958a Plant Physiol. 33, 17–22
Curtis R W 1958b Science 128, 3325, 661–662
Leelavathy K M 1969 Plant and Soil 30, 318–335
Narain A and Prakash O 1968 Indian Phytopath. 21, 217–220
Odamtten G T and Clerk G C 1980 Phytophthora Newsletter 8, 37
Odamtten G T and Clerk G C 1985 Proceedings 9th International Cocoa Res. Conf. Cocoa Producers' Alliance, Lagos 293–301
Postlethwait S N and Curtis R W 1959 Am. J. Bot. 4B, 31–35
Purvis M J *et al.* 1966 Laboratory Techniques in Botany. Butterworth and Co. London
Reiss J 1970 Phytopath. Z. 69, 78–82