Ann. appl. Biol. (1994), **124**, 173–178
Printed in Great Britain

In vivo use of extracts from *Ocimum gratissimum* and *Cymbopogon citratus* against *Phytophthora palmivora* causing blackpod disease of cocoa

By R T AWUAH

Department of Crop Science, University of Science and Technology (UST), Kumasi, Ghana

(Accepted 6 July 1993)

Summary

Crude steam distillate from *Ocimum gratissimum* sprayed onto infection courts on detached cocoa pods moments after inoculation with *Phytophthora palmivora* completely inhibited the pathogen and blackpod lesion development on 75% of the infection courts. Disease suppression obtained with the extract was comparable to that obtained with a 2% Kocide 101 suspension. In the field, the *O. gratissimum* extract also suppressed lesion development although to a significantly lower ($P = 0.05$) extent in comparison with Kocide 101. Blackpod lesion expansion rates of 3.80, 3.56, 2.71 and 0.78 cm/day, respectively, were associated with pods treated in the field with *C. citratus* extract, tap water, *O. gratissimum* extract and 2% Kocide 101. The extract from *Cymbopogon citratus* was also ineffective on detached pods. Sporangia of *P. palmivora* from sporulating blackpod lesions on both detached and non-detached pods lost their infectivity within 1 h of treatment with the *O. gratissimum* extract. This effect was superior to that obtained with Kocide 101. Fungitoxicity of the extract on pods, however, was lost within 3 h of application. Thus, despite its *in vivo* effectiveness as an eradicant, the *O. gratissimum* extract, in its present form, has limited utility as a protectant fungicide.

Key words: Plant extracts, fungitoxicity, *Phytophthora palmivora*, cocoa

Introduction

Extracts from *Ocimum gratissimum* L. and *Cymbopogon citratus* (D.C.) Stapf have previously been reported to possess fungitoxic properties *in vitro* (Awuah, 1989, 1990). The nature of fungal suppression observed indicated potential for the two plant extracts in controlling fungal plant diseases.

Most reported work on *in vivo* use of plant extracts against plant pathogens has focused on seed treatments (Dikshit, Dubey, Tripathi & Dixit, 1983; Asthana, Dixit, Tripathi & Dixit, 1989) and on prevention of post harvest decay (Aulakh & Grover, 1968; Grover & Aulakh, 1968). Studies on the use of a plant extract to control a fungal disease affecting aerial plant parts are lacking.

The present study attempts to utilise extracts from *O. gratissimum* and *C. citratus* to control blackpod disease of cocoa (*Theobroma cacao* L.) caused by *Phytophthora palmivora* Butl.

Materials and Methods

Preparation of plant extracts

Chopped fresh leaves (200 g each) of *O. gratissimum* and *C. citratus* were separately steam distilled with 600 ml tap water using a steam distillation apparatus. The volume of distillate during a 7-min period (after the first production) was collected and stored in the dark in sterilised 180 ml capped bottles and used within 24 h.

Maintenance of test fungus and inoculum production

An isolate of *P. palmivora* obtained from the Cocoa Research Institute of Ghana, Tafo was used. Stock cultures of the fungus were maintained on oat-meal agar (OMA: Tuite, 1969) in a refrigerator. Sub-cultures were made on fresh OMA and a mycelial piece from the resulting colony was used to inoculate a detached cocoa pod. The pod was incubated in a humidified transparent polyethylene bag on a laboratory bench for 7 days and mycelia-bearing sporangia of the fungus from the resulting blackpod lesion were employed as inoculum in subsequent experiments. Mature green pods of the Amelonado type cocoa were used in all experiments.

Disease suppression with plant extracts

This was done on both detached cocoa pods in a screenhouse and on pods which remained on the tree. In the former case, cork borer wells (7 mm diameter, 3 mm deep) were made on pods and pieces of sporangia-bearing mycelia were transferred into the wells. A 2 ml aliquot of each plant extracted was sprayed onto each infection court and the pod placed in a humidified, inflated transparent polyethylene bag in diffuse sunlight on a screenhouse bench. There were four replicate pods per extract. Tap water and 2% Kocide 101 suspension (77 WP; 50% metallic copper content) controls were also set up. Pods were monitored for blackpod lesions, the diameters of which were measured daily for 6 days. The field bioassay was conducted on pods borne on a tree in a planting belonging to the Grounds and Gardens Department, UST. A similar procedure to that described above was employed, except that treated pods were not enclosed in polyethylene bags.

Infectivity of sporangia treated with O. gratissimum *extract*

Three cocoa pods borne on the same tree were inoculated with *P. palmivora* as above and enclosed in humidified polyethylene bags to induce sporulation of the fungus on the resulting lesions. Each sporulating lesion was either sprayed with 5 ml of *O. gratissimum* extract, tap water or 2% Kocide 101 suspension. Pods were left uncovered for 60 min and sporangia from the lesions washed off with tap water, filtered through four layers of cheese cloth and quantified with a haemacytometer. 0.1 ml aliquots of sporangial suspension (25 000 sporangia/ml) from each treatment were obtained with an Eppendorf pipette and placed into three cork borer wells made on each of four pods on the same tree. The pods remained uncovered and were moistened with tap water spray after two days. Diameters of blackpod lesions were measured after a further two days. The experiment was repeated on detached pods in the screenhouse where four inoculations per pod were used.

Persistence of O. gratissimum *extract*

Cork borer wells were made on 10 cocoa pods (two wells, 5 cm apart per pod) and filled with 0.1 ml fresh extract of *O. gratissimum* using an Eppendorf pipette. Treatment with tap water and 2% Kocide 101 suspension served as controls. At 0, 3, 6, 9 and 12 h, pieces of sporangia-bearing hyphae of *P. palmivora* from a sporulating blackpod lesion were

transferred into the treated wells in 50 μl drops of tap water. The pods were incubated in humidified polyethylene bags on a screenhouse bench and diameters of the resulting blackpod lesions were measured after 3 days.

Results

Blackpod lesions developed on all inoculated detached and non-detached cocoa pods treated with *C. citratus* extract and lesion sizes on such pods were not significantly different ($P = 0.05$) from those associated with the tap water control (Figs 1A & 1B). On detached pods, the extract from *O. gratissimum* completely inhibited lesion development on three of the four pods, delaying lesion appearance on the fourth pod by four days (Fig. 1A). Fungitoxicity of the *O. gratissimum* extract on such pods was similar to that obtained with the 2% Kocide 101 suspension.

In the field, however, all four inoculated cocoa pods treated with the *O. gratissimum* extract developed blackpod lesions, though lesion initiation was delayed by 2 days (Fig. 1B). This is contrasted with Kocide 101 which, in the field, completely inhibited lesion development on three of the four pods and delayed lesion development on the fourth by three days (Fig. 1B). Blackpod lesion expansion rates of 3.80, 3.56, 2.71 and 0.78 cm/day, respectively, were calculated for *C. citratus* extract, tap water control, *O. gratissimum* extract and the 2% Kocide 101 suspension in the field test.

All tested sporangia from sporulating blackpod lesions on detached and non-detached field pods lost infectivity within 1 h on treatment with the *O. gratissimum* extract (Table 1). On treatment with the 2% Kocide 101 suspension, 87.5% of tested sporangia from detached pods and 58.3% from non-detached pods lost infectivity.

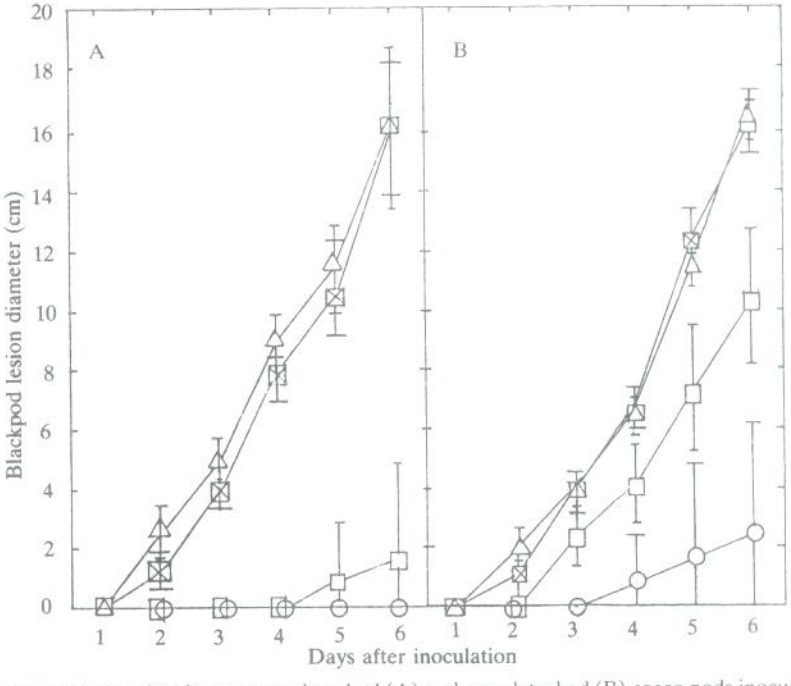

Fig. 1. Blackpod lesion development on detached (A) and non-detached (B) cocoa pods inoculated with *P. palmivora* and subsequently (—△—) treated with tap water (—⊠—), *C. citratus* extract, (—□—) *O. gratissimum* extract, and (—○—) 2% Kocide 101 suspension. Each point is the mean of four replications. Vertical bars indicate 2× SE of the means.

Table 1. *Infectivity of sporangia of* P. palmivora *from sporulating blackpod lesions treated with* O. gratissimum *extract and 2% Kocide 101 suspension**

Treatment	Detached pods No. diseased / No. treated	Detached pods Lesion diameter (cm) ± 2 SE	Field pods No. diseased / No. treated	Field pods Lesion diameter (cm) ± 2 SE
O. gratissimum	0/16	0 ± 0	0/12	0 ± 0
2% Kocide	2/16	0.82 ± 1.20	5/12	1.03 ± 0.58
Tap water (control)	16/16	5.74 ± 0.22	12/12	4.81 ± 0.70

*Data was taken after 4 days; lesion diameters on detached and field pods are the means of 16 and 12 replications, respectively.

Table 2. *Blackpod lesion diameters (cm) on detached cocoa pods treated with* O. gratissimum *extract and thereafter inoculated at 3-h intervals with* P. palmivora*

Treatment	Hours after treatment 0 Lesion diameter ± 2 SE	3 Lesion diameter ± 2 SE	6 Lesion diameter ± 2 SE	9 Lesion diameter ± 2 SE	12 Lesion diameter ± 2 SE
O. gratissimum	0 ± 0	5.18 ± 0.40	5.06 ± 0.44	5.03 ± 0.36	5.00 ± 0.24
Kocide 101	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Tap water (control)	5.76 ± 0.22	5.63 ± 0.26	5.10 ± 0.52	5.66 ± 0.38	5.30 ± 0.62

*Data was taken after 3 days. Each lesion diameter is the mean of four replications.

Fungitoxicity of the *O. gratissimum* extract was lost within 3 h after application to pods (Table 2). Kocide 101, however, maintained its activity during the 12 h test period.

Discussion

Although plant extracts have been used for seed dressing (Chandra, Mall & Tripathi, 1982; Dikshit *et al.*, 1983; Dubey, Bhargava & Dixit, 1983; Asthana *et al.*, 1989) and for reducing post harvest decay of vegetables (Aulakh & Grover, 1968; Grover & Aulakh, 1968), information on a plant extract being used as a fungicide spray is rare. In the present study, extracts from *O. gratissimum* and *C. citratus* have been utilised for *in vitro* application against a plant pathogen. Of the two extracts tested, that from *O. gratissimum* proved more promising in suppressing blackpod disease of cocoa. On detached pods, this extract compared favourably with Kocide 101, a protectant fungicide commonly utilised for blackpod disease control in Ghana. On such pods, the *O. gratissimum* extract completely suppressed *P. palmivora* and blackpod lesion development in 75% of the cases, confirming its fungicidal nature (Tripathi *et al.*, 1986; Awuah, 1990). The extract from *C. citratus* was inferior as a fungitoxicant on cocoa pods despite its proven fungitoxic properties *in vitro* (Gyane, 1976; Awuah, 1989, 1990). Gyane (1976) alluded to the antifungal nature of citral which is the major component of *C. citratus* extract (Todd, 1967; Trease & Evans, 1983;

Purseglove, 1972). Due to its extreme volatility and photolability, extract from the plant is recommended for storage in airtight containers and away from light (Reynolds & Prasad, 1977). Thus, rapid loss of the citral and consequently of the fungitoxicity of the *C. citratus* extract would be expected to occur when the extract is sprayed onto pods.

The extract from *O. gratissimum* was more effective than Kocide 101 in killing sporangia of *P. palmivora* on cocoa pods. Sporangia of the fungus (and zoospores formed from them) on infected cocoa pods serve as inocula in perpetuating secondary cycles of blackpod disease of cocoa (Lass, 1985). Thus, from the epidemiological point of view, inactivation of sporangia present on diseased cocoa pods is desirable. The present study, however, did not determine how long after treatment with the extract it takes for another crop of infectious sporangia to form. This information could form the basis for determining the frequency of applying the *O. gratissimum* extract to sporulating blackpod lesions.

Although the *O. gratissimum* extract was very effective as a spray on detached cocoa pods, its fungitoxicity was much reduced in the field where treated pods were not enclosed in polyethylene bags after treatment. This indicates that like the *C. citratus* extract, the active principle in the *O. gratissimum* extract is degradable on exposure to the elements, albeit to a lesser extent. It was determined in the present study that the fungitoxicity of the *O. gratissimum* extract on cocoa pods was lost within 3 h of application.

The fungitoxic principle in *O. gratissimum* has not been determined with certainty. Tripathi *et al.* (1986) reported eugenol to be the major and most biologically active constituent of *O. gratissimum*. They did not indicate presence of thymol in the plant. Sainsbury & Sofowora (1971), however, did not detect eugenol in the Nigerian *O. gratissimum* but rather thymol which they noted to be the major component. Earlier, El-Said, Sofowora, Malcolm & Hofer (1969) reported this and speculated that thymol could be the most biologically active constituent in *O. gratissimum*. Further studies are thus needed to conclusively characterise the active principle in the Ghanaian *O. gratissimum* to provide the basis for studies aimed at enhancing its stability. Until this is accomplished, the *O. gratissimum* extract, in its present form, will have limited utilisation as a protectant fungicide, despite its efficacy as an eradicant of fungal propagules present on aerial plant parts. Because of its eradicant nature, the potential of the crude *O. gratissimum* extract for seed treatment and for preventing pathological post harvest decay of fruits and vegetables appears good and should be investigated.

Acknowledgements

This research was fully supported by a research grant from the International Foundation for Science (IFS), Sweden.

References

Asthana A, Dixit K, Tripathi N N, Dixit S N. 1989. Efficacy of *Ocimum* oil against fungi attacking chilli seeds during storage. *Tropical Science* **29**:15–20.

Aulakh K S, Grover R K. 1968. Ripe fruit rots of tomato and their control by oils. *Plant Disease Reporter* **52**:555–559.

Awuah R T. 1989. Fungitoxic effects of extracts from some West African plants. *Annals of Applied Biology* **115**:451–453.

Awuah R T. 1990. Fungitoxicity spectra of extracts from three Ghanaian plants. *International Seminar on Natural Products Research and Development of Plant Based Drugs, U.S.T. Kumasi. Book of Abstracts*, 46 pp.

Chandra H, Mall H V, Tripathi N N. 1982. Protectant activity of *Ageratum* oil against fungal deterioration of chilli seeds during storage. *Journal of Indian Botanical Society* **6**:19.

Dikshit A, Dubey N K, Tripathi N N, Dixit S N. 1983. Cedrus oil – a promising storage fungitoxicant. *Journal of Stored Product Research* **19**:159–162.

Dubey N K, Bhargava K S, Dixit S N. 1983. Protection of some stored food commodities from fungi by essential oils of *Ocimum canum* and *Citrus medica*. *International Journal of Tropical Plant Diseases* **1**:177–179.

El-Said F, Sofowora E A, Malcolm S A, Hofer A. 1969. An investigation into the efficacy of *Ocimum gratissimum* as used in Nigerian native medicine. *Plant Medica* **17**:194–200.

Grover R K, Aulakh K S. 1968. *Oospora* rot of tomato fruits and its control by oils. *Indian Phytopathology Society Bulletin* **4**:37–42.

Gyane D O. 1976. Preservation of Shea butter. *Drugs and Cosmetics Industry* **188(5)**:36–38;138–140.

Lass R A. 1985. Diseases. In *Cocoa*, 4th Edition, pp. 165–365. Eds G A R Wood and R A Lass. Wood Tropical Agriculture series. Essex, England: Longman. 620 pp.

Purseglove J W. 1972. *Tropical Monocotyledons*, Volumes 1 & 2 combined. Essex, England: Longman. 607 pp.

Reynolds E F, Prasad A B. 1977. *The Extra Pharmacopoeia*, 27th Edition. London: The Pharmaceutical Press. 2077 pp.

Sainsbury M, Sofowora E A. 1971. Essential oil from the leaves and inflorescence of *Ocimum gratissimum*. *Phytochemistry* **10**:3309–3310.

Todd R G. 1967. *The Extra Pharmacopoeia*, 25th Edition. London: The Pharmaceutical Press. 1804 pp.

Trease G E, Evans W C. 1983. *Pharmacognosy*, 12th Edition. Eastbourne, UK: Bailliere Tindall. 812 pp.

Tripathi R D, Banerji R, Sharma M L, Balasubrahmanyan V R, Nigam S K. 1986. Toxicity of *Ocimum gratissimum* (clocimum) against betelvine pathogenic fungi. *Horticultural Abstracts* **56**:288.

Tuite J. 1969. *Plant Pathological Methods, Fungi and Bacterial*. Minneapolis, Minnesota, USA: Burges Publishing Company. 239 pp.

(Received 26 January 1993)