J. Mycopathol. Res. **40(1)** : 51-55 (2002) *Printed in India*

Gaseous hydrocarbon production by fungi and responses of a triazole (BAS III...W)

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Six fungi were isolated from banana (*M. acuminata* L. cv. Giant governor) stored under high relative humidity. Within five hours of subculturing fungi produced ethylene, ethane and acetylene in different concentration which decreased with culture age. Triazole inhibited such production. Ethylene production was substantially low in healthy noninfected zone of banana.

Key words : Banana, fungi, ethylene (C_2H_4), ethane (C_2H_6), acetylene (C_2H_2), triazole (Bas III...W)

INTRODUCTION

The production of ethylene by isolated fungi and by diseased plants have been previously reported (Fergus, 1954; Ilag and Curtis, 1968; Chalutz and Devay, 1968; Dasilva *et al.* 1974; Vasyuk, 1995; Sadhu and Gupta 1996). A possible role which ethylene may play in host-parasite interactions was presented by Freebairn and Buddenhagen (1994) in a study on banana wilt disease caused by *Pseudomonas solanacearum*. Stahmann *et al.* (1966) reported ethylene induced resistance to black rot in sweet potato. However, the involment of ethylene in pathogenecity is controversial (Chalutz *et al* .1968; Dasilva *et al.*1974; Popp *et al.* 1995). Extensive study is therefore needed on the production of ethylene by the pathogens under *in vitro* condition and the extent of ethylene production by the host-parasite complex.

This investigation was carried out with the fungi (*Aspergillus flavus, Botryodiplodia theobromae, Curvularia fallax, Fusarium oxysporum, F. moniliforme, Penicilium citrinum*) isolated from banana in order to observe the capacity of the pathogens to produce gaseous hydrocarbons with progress of growth under cultural conditions. Ethylene production rate has also been studied in host – parasite region of banana. The amount of ethylene production has been recorded in infected zone, healthy zone and intermediate zone (between healthy and infected zone) of coat and pulp tissues of banana. In addition the responses of a triazole derivative were evaluated on the gaseous hydrocarbon production *in vitro* (Sadhu, 1994). Responses of triazole on inhibition of ethylene production has been reported in higher plants (Grossman et al., 1989). However, such studies have been made in fungi where the methionine pathway of ethylene production is suspected to be not operative.

MATERIALS AND METHODS

Experiments were performed on fungi isolated from Banana Research Station, Chinsura, Hooghly, W.B. they were surface sterilized and stored in high relative humidity at 30 ±1°C until fungi became visible. Fungi were isolated from stalks, coat and pulp tissues of banana. Isolated fungi were grown on potato dextrose agar (PDA) medium (pH 6.5) at 30 ± 1°C in dark. They were identified by Commonwealth Mycological Institute (CMI, U.K.) and Indian Agricultural Research Institute (India). Pathogenecity tests were carried out with conidia from 6 day old culture which resulted in the development of typical symptom 6 – 8 days after inoculation.

An inoculum plug (5 mm diameter) was transferred to PDA slants containing 8 ml of media in borosil culture tube (15.2 cm × 1.6 cm) and incubated at 30 ± 1°C in the dark. Fresh weights were taken at 0 and 10 days after the slants were inoculated by weighing the control slants and inoculated slants side by side. Ethylene, ethane and acetylene production were measured from the slant cultures (5h and 10 d) using the method described by Spalding and Libermann (1965). Cultures tubes were inoculated and sealed with rubber serum cap aseptically and incubated at 26 ± 1° C in dark for 5 hours. Gas samples were withdrawn with a

Hamilton (T : M) gas tight syringe and analysed in a Nucon 5700 gas chromatograph fitted with Porapak T column ($\frac{1}{8}$ ", mess 80 - 100) on a flame ionization detector. Carrier flow (N_2) and oven temperature were maintained at 30 ml/min and 35 ± 1° C respectively to determine concentrations of ethylene, ethane and acetylene peak. Standard gaseous mixtures were obtained from EDT Research Co. (U.K.). Ethylene, ethane and acetylene production were also measured in 10 day old perturbed mother culture.

Ethylene production rate was measured in healthy, infected and intermediate zones of pulp and coat tissues of banana. Banana was stored in room temperature (winter months) and pulp (10 mm × 5 mm) and coat (5 mm × 1.5 mm) discs were transferred in 5 ml capacity glass vial fitted with latex septum. It was then incubated at 30 ± 1° C in dark. Ethylene production rate was measured at 5 hours and 24 hours of incubation during storage as described above. A triazole derivative namely BAS 111 W [1-phenoxy-3-(1 H-1,2,4-triazole-1-yl)-4-hydroxy 5, 5-dimethyl hexane] from BASF, Germany were mixed with PDA slants making 100 µg/ml (Sadhu and Gupta, 2000) which inhibits fungal growth. The slants were inoculated with the test fungi (5 ml discs from active colonies) and levels of ethylene, ethane and acetylene were measured after 5 hrs and 10 days as outlined previously.

RESULTS AND DISCUSSION

In the present study ethylene production from healthy and infected region of banana during storage was followed (Table 1). The possible role of ethylene in host-parasite interactions of fruits after harvest is of scientific interest and great commercial significance. There is, however, little information available about the effect of ethylene on disease development. The infected coat of banana which has dried up, produced very little amount of ethylene. Intermediate zone (i.e. between healthy and infected zone of coat and pulp) produced more ethylene than infected zone. Infected pulp zone produced more ethylene than healthy pulp. It might be due to the presence of fungi in infected zone which grow towards healthy zone (Achilea *et al.*, 1985; Sadhu and Gupta, 1996). So the fungi present in the intermediate zone became more active in ethylene production. There was no direct relationship between the amount of mycelium formed and the amount of ethylene produced (Fergus, 1954; Vasyuk, 1995). On the other hand Chalutz and Devay (1968) in their studies with *Ceratocystis fimbriata* found that ethylene production was dependent upon the rate and amount of fungal growth on agar media and in host tissue but there was no consistent relationship between the relative amount of ethylene produced *in vivo* and *in vitro*.

Table 1. Ethylene production from banana (*M. acuminata*) coat and pulp tissues (infected zone towards healthy zone) stored in room temperature (winter months) at different hours of incubation during storage.

	Ethylene (nl ml gm)			
	2d		4d	
Zone	5 hrs	24 hrs	5 hrs	24 hrs
Healthy coat	3.12 ±0.05	4.90 ±0.06	4.65 ±0.05	6.10 ±0.07
Infected coat	0	0.82 ±0.01	0.96 ±0.02	1.10 ±0.04
Intermediate zone between healthy and infected coat	11.21 ±0.15	13.83 ±0.21	21.87 ±0.25	25.70 ±0.30
Healthy pulp	5.00 ±0.08	5.96 ±0.08	7.23 ±0.12	9.18 ±0.16
Infected pulp	36.21 ±0.42	39.42 ±0.46	40.20 ±0.48	42.85 ±0.45
Intermediate zone between healthy and infected zone	58.50 ±0.65	62.12 ±0.72	72.56 ±0.91	88.31 ±0.93

(Values are mean ± S.E.)

Mycelial growth in terms of fresh weight was followed 10 days after inoculation and it was observed that maximum growth was attained by *Botryodiplodia theobromae* followed by *Fusarium oxysporum*, *Curvularia fallax*, *Aspergillus flavus* and *Fusarium moniliforme*. *Penicillium citrinum* showed slow rate of growth. Gaseous hydrocarbon production was followed with the increment of culture fresh weight in the same system and time. It was observed from 5 hours to the 10 days and most of the fungi produced ethylene, ethane and acetylene (Table 2). The amount of ethane production was in general very low exept *Aspergillus flavus* and *Fusarium moniliformae*. It was also observed that production of ethane and acetylene were highest where ethylene production was maximum in the same system. Maximum levels of all three hydrocarbons were at the 5 h assessment stage.

Table 2. Gaseous hydrocarbon (ethylene, ethane and acetylene) production from fungsl cultures (30°C in dark) at 5 hr. and 10 days following inoculation

Hydrocarbon (nl ml)	Age (hr days)	Control	Fungi					
			A. flavus	*B. theobromae*	*C. fallax*	*F. oxysporum*	*F. moniliforme*	*P. citrinum*
Ethylene	5 h	0	1372.00 ± 8.15	657.81 ± 4.18	119.00 ± 1.88	33.12 ± 0.72	1281.00 ± 7.45	159.11 ± 1.87
	10 d	0	6.25 0.22	8.60 ± 0.29	1.25 ± 0.09	7.52 ± 0.12	7.48 ± 0.11	7.82 ± 0.12
Ethane	5 h	0	80.00 ± 0.93	35.10 ± 0.20	6.85 ± 0.10	0	114.70 ± 1.72	9.47 ± 0.29
	10 d	0	0	0	0	0	0	0
Acetylene	5 h	0	668.95 ± 5.21	359.10 ± 2.80	78.66 ± 0.90	13.65 ± 0.28	779.80 ± 6.40	78.15 ± 0.12
	10 d	0	0.82 ± 0.01	1.12 ± 0.08	0	0.41 ± 0.01	0	2.90 ± 0.96

(Values are mean ± S.E.)

then decreasing as the culture aged. In *A. flavus* and *C. fallax* ethylene production was almost nil at 10 th day and ethane and acetylene production was not found. In other cultures hydrocarbon production rate was very low at 10 th day. Ethylene and acetylene production was maximum in *A. flavus* followed by *F. moniliformae*. *B. theobromae*. *P. citrinum* and *C.fallax*. *F.oxysporum* produced minimum amount of gaseous hydrocarbon (Table 2).

Table 3. Production of gaseous hydrocarbon (ethylene, ethane and acetylene) from the perturbed mother culture (10 days old)

Fungi	Hydrocarbon (nl ml)		
	Ethylene	Ethane	Acetylene
A. flavus	687.03 ±4.23	12.89 ±0.24	106.70 ±1.26
B. theobroamae	128.73 ±1.31	7.20 ±0.50	42.80 ±0.81
C. fallax	29.92 ±0.42	1.20 ±0.05	4.92 ±0.08
F. oxysporum	9.25 ±0.70	0	0
F. moniliforme	438.23 ±3.02	22.42 ±0.37	72.30 ±1.08
P. citrinum	28.40 ±0.33	0	3.42 ±1.36

(Values are mean ± S.E.)

The present study showed that the highest ability for gaseous hydrocarbon production appeared to be immediately after the transfer of inocula in the media i.e. only after 5 hrs. of incubation. The biosynthesis of stress ethylene in response to mechanical wounding has been variously reported (Willamson, 1950; Hyodo and Nishino, 1981; Hoffman and Yang, 1982; Vasyuk, 1995; Sadhu and Gupta, 1996). Inoculum during transfer suffers mechanical wounding at the edge which could act as an induction for ethylene production (Table 3). This subsequently decreased after initial wound response. Results from this study are consistent with the idea that the physiological stress resulting from mechanical removal of the inoculum from the original culture (Table 3) resulted in an elevated rate of ethylene production. Here in case of *A. flavus* highest quantity of ethylene and acetylene was noted. On the other hand in case of *C. fallax*. *F. oxysporum* and *P. citrinum* such production was significantly low when compared with that of *A. flavus*. But *B. theobromae* produced moderate amount in comparison to others (Table 2). In addition to ethylene the fungi have the ability to produce ethane and acetylene.

Triazoles have been reported to have fungicidal properties (Ali *et al*., 1979; Fletcher *et al*., Kar and Gupta, 1991; Sadhu and Gupta, 2000). irrespective of weather they are released as fungicides or plant growth regulators. They effectively inhibit GA, sterol and ethylene biosynthesis (Grossman *et al*., 1989). Triazole (BAS lll..W) reduced ethylene, ethane and acetylene production in all the test fungi (Table 4). Treatment with BAS lll..W have been shown to decrease ethylene production but increased l amino cyclopropane carboxylic acid (ACC)

Table 4. Triazole (BAS 111.W at 100 µg ml⁻¹) responses on gaseous hydrocarbon (Ethylene, ethane and acetylene) production from fungi at 5 hr. and 10 th day of inoculation

Fungi	Time after Inoculation (h d)	Ethylene (nl ml)		Ethane (nl ml)		Acetylene (nl ml)	
		Control	Treated	Control	Treated	Control	Treated
A. flavus	5 h	1372.00 ±8.15	257.50 ±2.05	80.00 ±0.93	12.15 ±0.33	668.95 ±5.21	168.00 ±0.97
	10 d	6.25 ±0.22	5.46 ±0.23	0	0	0.82 ±0.01	0
B. theobromae	5 h	657.81 ±4.18	296.20 ±1.85	35.10 ±0.20	16.70 ±0.10	359.10 ±2.80	179.50 ±1.39
	10 d	8.60 ±0.29	4.91 ±0.16	0	0	1.12 ±0.08	0
C. fallax	5 h	119.00 ±1.88	90.00 ±1.42	6.85 ±0.10	5.10 ±0.07	78.66 ±0.90	60.00 ±0.45
	10 d	1.25 ±0.09	1.25 ±0.25	0	0	0	0
F. oxysporum	5 h	33.12 ±0.72	8.75 ±0.40	0	0	13.65 ±0.28	4.20 ±0.08
	10 h	7.52 ±0.12	2.23 ±0.05	0	0	0.41 ±0.01	0
F. moniliforme	5 h	1281.00 ±7.45	905.72 ±5.10	114.70 ±1.72	92.40 ±1.30	779.80 ±6.40	543.20 ±4.32
	10 d	7.48 ±0.11	1.20 ±0.02	0	0	0	0
P. citrinum	5 h	159.11 ±1.87	137.50 ±1.31	9.47 ±0.29	7.08 ±0.21	78.15 ±1.12	84.00 ±1.20
	10 d	7.82 ±0.12	2.50 ±0.02	0	0	2.90 ±0.03	0

(Values are mean ± S.E.)

production in leaf discs of barley (Grossman *et al.*, 1989). Triazoles in moderately low concentration (300 µg/ml) increased storage life of banana (Sadhu, 1994). Role of gaseous hydrocarbons in disease development and their protection by triazoles are under trial.

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(Accepted for publication February 28, 2002)