

Different ethylene production in vitro by several species and formae speciales of *Fusarium*

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Abstract

The ethylene production of several *Fusarium* species and formae speciales in vitro was measured under comparable conditions. All of them produced ethylene. *Fusarium oxysporum* Schlecht. ex Fr. f. *tulipae* Apt. produced much more than the other 18 strains investigated.

Introduction

Ethylene is involved in the development of some fungal and bacterial diseases (e.g. Freebairn and Buddenhagen, 1964; Stahmann et al., 1966; Sakai et al., 1970; Beijersbergen and Bergman, 1973). Some micro-organisms produce ethylene under pure culture conditions (Ilag and Curtis, 1968; Bird and Lynch, 1974), others only in host tissue (Lund and Mapson, 1970; Imaseki et al., 1968). The rate of the ethylene production by various organisms in vitro can vary widely, and single-spore isolates of the same pathogen can also differ greatly in this respect (Spalding and Lieberman, 1965; Chalutz and DeVay, 1969).

Studies on the cause of some physiological disorders in tulips have shown that ethylene produced by *Fusarium oxysporum* f. *tulipae* is a factor in the genesis of bud necrosis, flower-bud blasting, gummosis, root and shoot inhibition, and other aberrations seen in tulips (Kamerbeek and de Munk, 1976). This led us to investigate factors influencing in vitro ethylene production by the tulip *Fusarium* (Swart and Kamerbeek, in preparation). A comparative study on the in vitro ethylene production of a number of *Fusarium* species and formae speciales is reported here.

Material and methods

Conidia were collected from several 4-day-old *Fusarium* cultures on potato-dextrose agar. A conidium suspension consisting of about 250000 conidia in 1 ml distilled water was used as inoculum in a modified Pratt medium composed of mineral salts, glucose, and yeast extract (Spalding and Lieberman, 1965). Erlenmeyer-shaped suction flasks (250 ml) were provided with 50 ml of the medium. After inoculation, the openings at the top and the side of the flasks were sealed with serum caps and the flasks were placed in the dark at 25°C under constant shaking (125 rotations per minute). For ethylene analysis, 1 ml air samples were taken with a syringe daily after which the atmosphere in the flasks was flushed with fresh sterile air, at least three

times the volume. The total production of ethylene during a 15-day period was estimated by adding up the amounts produced daily. This duration was chosen to make certain that the period of maximum ethylene production was included. Further details will be given elsewhere (Swart and Kamerbeek, in preparation).

The Centraal Bureau voor Schimmelcultures (CBS) at Baarn kindly supplied most of the *Fusarium* cultures. Other cultures, indicated as LBO, were made with strains isolated by colleagues in our institute. A highly virulent strain of *Fusarium oxysporum* f. *lycopersici* was obtained from the Glasshouse Crops Research and Experimental Station, Naaldwijk.

Results and discussion

The amount of ethylene produced during the 15-day period and the ultimate dry weight of the mycelium after 15 days of growth are given in Table 1 (mean values of 4 replicates). The values for ethylene production and the ultimate dry weight of the mycelium are given separately because it was found, at least for the tulip *Fusarium* isolates used, that the production per unit weight of mycelium was not a valid referential base. First of all, the peak production of ethylene occurred at the end of the growth phase of the mycelium; production and growth (increment in weight) were not correlative. Secondly, some factors, e.g. the oxygen tension, were found to influence production and growth to a different degree (Swart and Kamerbeek, in preparation).

The total production of ethylene in uninoculated culture media (controls) did not exceed 0.04 μg in the 15-day period. The most striking result is the exceptionally high ethylene production by the *Fusarium* pathogenic to tulips as compared with all other *Fusarium* strains tested. The production of the tulip *Fusarium* varied from about 600 to 4200 μg with an average of 2300 μg in 15 days, whereas the other species and formae speciales produced 0.13 to 0.79 μg ethylene with an average of 0.45 μg in 15 days. It may be concluded that all species and formae speciales of *Fusarium* produce ethylene but that only *Fusarium oxysporum* f. *tulipae* produces very high quantities (up to 5000 times more), although differences were found between the various isolates. The high ethylene production of the tulip *Fusarium*, also found in vivo (de Munk, 1972) must be taken into account in the investigation of host-parasite interactions, because ethylene can influence many processes in tulips (Kamerbeek and de Munk, 1976). The finding that the synthesis of tuliposid, regarded as a barrier against infection by *Fusarium* in the scale tissue of tulips, is suppressed by ethylene, is of interest in further studies of the host-parasite relation (Beijersbergen and Bergman, 1973).

Differences between the ethylene production of various isolates of the tulip *Fusarium* may be related with the virulence of these isolates (Table 1), but testing of differences in virulence was technically difficult (Bergman, pers. communication). This study is also complicated by the unexplained fact that the total production of ethylene showed a rather great variation when experiments with the same strain are repeated (Table 1). Probably this may be attributed at least in part to the rate of oxygen consumption by the growing mycelium, because oxygen tension greatly influences ethylene production (Swart and Kamerbeek, in preparation).

Table 1. Ethylene production and mycelium growth of various *Fusarium* species and formae speciales.

	Total C ₂ H ₄ production over a 15-day period (in µg)	Dry weight mycelium after 15 days (in mg)	Origin and number of culture
<i>F. sulphureus</i> Schlecht. ex Fr.	0.40	205.1	CBS 185.35
<i>F. moniliforme</i> Sheldon	0.48	207.8	CBS 240.64
<i>F. culmorum</i> (W. G. Sm.) Sacc. var. <i>cereale</i> (Cke) Wollenw.	0.79	187.4	CBS 251.52
<i>F. avenaceum</i> (Fr.) Sacc. f. 1 Wollenw.	0.46	184.4	CBS 170.31
<i>F. redolens</i> Wollenw. f. sp. <i>dianthi</i> Gerlach	0.44	277.6	CBS 248.61
<i>F. sporotrichioides</i> Sherb.	0.49	141.9	CBS 180.32
<i>F. poae</i> (Peck) Wollenw.	0.79	174.0	CBS 130.24
<i>F. merismoides</i> Corda var. <i>crassum</i> Wollenw.	0.77	259.2	CBS 180.31
<i>F. solani</i> (Mart.) Sacc. var. <i>minus</i> Wollenw.	0.41	277.2	CBS 204.31
<i>F. solani</i> (Mart.) Sacc. var. <i>minus</i> Wollenw.	0.29	256.5	CBS 208.29
<i>F. camptoceras</i> Wollenw. & Reinking	0.74	166.2	CBS 193.65
<i>F. oxysporum</i> Schlecht. ex Fr. f. sp. <i>lycopersici</i> (Sacc.) Snyder & Hansen	0.59	218.0	CBS 249.52
<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	0.20	210.6	Naaldwijk
<i>F. oxysporum</i> from <i>Fritillaria</i>	0.55	230.4	LBO
<i>F. oxysporum</i> f. sp. <i>narcissi</i> Snyder & Hansen	0.21	219.3	LBO
<i>F. oxysporum</i> from <i>Hippeastrum</i>	0.18	323.2	LBO
<i>F. oxysporum</i> from <i>Hyacinth</i>	0.21	313.0	LBO
<i>F. oxysporum</i> from <i>Freesia</i>	0.13	239.6	LBO
<i>F. oxysporum</i> Schlecht. ex Fr. f. sp. <i>tulipae</i> Apt. 1223		212.7	CBS 195.65
<i>F. oxysporum</i> Schlecht. ex Fr. f. sp. <i>tulipae</i> Apt. 1726		219.2	CBS 242.59
<i>F. oxysporum</i> f. sp. <i>tulipae</i>	3678	261.5	LBO B.1 ²
	3098	271.6	
	4231	190.4	
	2231	229.5	
	3294	236.5	
<i>F. oxysporum</i> f. sp. <i>tulipae</i>	3168	209.0	LBO B.2 ²
	1612	252.6	
<i>F. oxysporum</i> f. sp. <i>tulipae</i>	2959	229.1	LBO B top 4 ²
<i>F. oxysporum</i> f. sp. <i>tulipae</i>	1762	210.0	LBO A on 3 ¹
	565	228.1	
<i>F. oxysporum</i> f. sp. <i>tulipae</i>	565	220.6	LBO A ap 27 ¹

¹ Less virulent.

² Highly virulent.

Tabel 1. Ethyleen-productie en mycelium-groei van verschillende *Fusarium*-soorten en formae speciales.

Samenvatting

Verschillen in ethyleenproductie in vitro door diverse soorten en formae speciales van *Fusarium*

Aangezien er aanwijzingen zijn dat ethyleen een rol speelt bij de aantasting van de tulpebol door *Fusarium*, werd de ethyleenproductie van diverse soorten en formae

speciales van *Fusarium* onder standaardvoorwaarden in vitro vergeleken. Alle onderzochte *Fusarium* spp. en vormen van *Fusarium* produceerden ethyleen. *Fusarium oxysporum* Schlecht. ex Fr. f. *tulipae* Apt. bleek in vergelijking met 18 andere getoetste soorten en formae speciales enige duizenden malen meer ethyleen te produceren.

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