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Ethylene not responsible for inhibition of conidium germination by soil volatiles

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Abstract

Volatile factors inhibiting the germination of *Botrytis cinerea* and *Cochliobolus sativus* conidia on membrane filters were emanated from six alkaline and two acid soils of different organic content and origin. Addition of lime or chitin increased the inhibitory action. Evidence is presented that ethylene is not involved either directly or indirectly in the inhibition of conidium germination above the different soils tested.

Introduction

Mycostatic effects of soil have been explained by microbial competition for nutrients needed for germination of fungal conidia and by the presence of volatile and non-volatile inhibitors. The importance of soil mycostasis for survival of plant pathogens in soil, has stimulated research in this field, especially over the last 10 years. A critical review of the subject has recently been presented by Lockwood (1977). Ethylene (Balis, 1976; Smith, 1973) has been reported as a volatile involved in soil mycostasis. It is regarded as a normal constituent of the soil atmosphere at concentrations ranging from less than 1 ppm to about 2 ppm, and exceptionally as high as 10 ppm (Smith and Restall, 1971; Rovira and Vendrell, 1972; Primose and Dilworth, 1976). Smith (1976) not only ascribes soil mycostasis to ethylene but also attributes to it a central role in a self-regulating microbial cycle in the soil, with important implications on conservation of organic matter and nitrogen and the control of soilborne plant pathogens. Smith (1973) noted that more ethylene was present in soils rich in organic matter than in those of low organic content. Release of volatile inhibitors have been shown to be most pronounced from alkaline soils (Hora and Baker, 1974) and to be stimulated by adding lime (Hora and Baker, 1975) or chitin (Schippers and Palm, 1973).

The present paper describes mycostatic effects caused by volatile inhibitors released from soils of different origin, pH and organic matter content and after additions of lime or chitin. The possible role of ethylene in these processes is analysed and discussed.

Material and methods

Origin and treatment of soils. Ten soils of different origin, pH and organic matter content were used (Table 1). River sandy loam originated from agricultural fields near Lienden (Schippers and de Weijer, 1972). Clay-loam soils, Flevo 1–4 were collected from agricultural field in the 'Zuid-Flevoland Polder'. Clay-loam soils WW and KA were collected at the experimental farms at Nagelen, Noord-Oost Polder. The KA fields have received inorganic fertilizers only for the last 25 years, the WW fields mainly organic manure. Gerendal soil is a calcareous loessoid clay (rentzina-like) collected from chalk grassland in the nature reserve Gerendal in the province of Limburg. The mull and mor soils originate from the A-Horizons in beach-birch and pine forest stands, respectively, at Baarn. All soils, except the Lienden soils, were stored in large plastic bags at 4 °C for a maximum of four months. The Lienden soil was sown with wheat during storage in the greenhouse at 16–19 °C. Wheat plants were removed after four to six weeks and about 4 weeks before the soil was used for experiments (Van Vuurde and Schippers, 1975).

One week before experimental use soils were sieved through a 3 mm mesh sieve,

Table 1. Inhibition of germination of conidia of *Botrytis cinerea* and *Cochliobolus sativus* directly exposed to the atmosphere above soil samples of different origin, pH, organic matter content or additions. Inhibition is expressed as percentage of germination above sterile pure sand (controls)[1] after 24 h.

Soils			Germination in % of control[1]	
origin and addition	pH	organic matter content	*B. cinerea*	*C. sativus*
Lienden	7.4	4.1	*68*[2] (86)	98 (96)
Lienden + chitin	7.4	5.1	*0* (74)	70 (86)
Lienden + lime	8.1	4.1	*9* (53)	*60* (89)
Flevo 1	7.5	10.0	*53* (95)	*79* (100)
Flevo 2	7.5	6.4	94 (95)	*62* (100)
Flevo 3	7.6	6.3	89 (95)	*69* (100)
Flevo 4	7.3	9.6	*71* (95)	*83* (100)
WW	7.2	2.6	*35* (90)	–[3]
KA	7.3	2.2	*46* (90)	–
Gerendal	7.5	12.0	*9* (83)	*88* (98)
Mull	3.4	18.0	*37* (83)	94 (98)
Mor	2.9	48.0	*11* (83)	*78* (98)

[1] % of germination of controls in parenthesis.
[2] The figures in italics indicate significant inhibitions ($p < 0.05$).
[3] No observations.

Tabel 1. Remming van de kieming van conidiën van Botrytis cinerea en Cochliobolus sativus boven grondmonsters van verschillende oorsprong, pH, organisch stofgehalte of verrijkt met chitine of kalk. De kiemremming is weergegeven als percentage van de kieming boven steriel zuiver zand (controle)[1] *na 24 uur.*

adjusted to about 25% water content (dry weight basis) and incubated at 20°C. Samples of Lienden soil were either amended with chitin (K & K Labs., 1000 ppm) one week before use in experiments, or with lime ($CaCO_3$:$Ca(OH)_2$ 1:1, 2.5 mg/g dry soil 24 h in advance. Soil pH was measured in 0.01 M $CaCl_2$ (Schofield and Taylor, 1955). Water-saturated pure sand, that had been heated to 300°C for 3 h, or Whatman filter paper saturated with a weak buffered salt medium, pH 5.7 (BM) after Griffin (1970) were used as controls.

Spore germination assays. Eight to ten-day-old cultures of *Botrytis cinerea* Pers. ex Nocca & Balbis and of *Cochliobolus sativus* (s. Ito & Kurib.) Drechsler ex Dastur (syn.: *Helminthosporium sativum* Pamm., King & Bakke) were irrigated with BM and the resulting conidium suspension was washed twice. Conidium suspensions (about 5.10^4 conidia/ml BM) were sprayed onto Sartorius membrane filters (2.0 μm, 25 mm diam.) with a chromatography atomizer. Membrane filters were halved and each half was adhered to a sterile glass slide with a drop of BM. The conidia were then incubated in 37 ml serum flasks with either 15 g soil, 15 g sterile water-saturated sand (control), or BM-saturated sterile Whatman filter paper (control), at 20°C. Serum flasks were sealed with rubber stoppers covered with aluminium caps during the incubation period (Fig. 1). Membrane filters with conidia were sampled either at intervals over a 30 h period or once after 24 h of incubation. They were air-dried, mounted on glass slides and stained with cotton blue in lactophenol. Germination was assessed for at least 100 conidia per flask.

Ethylene treatment of conidia and soils. Ethylene was injected into the serum flasks with a 5 ml Precision Sampling Pressure Lok A gas syringe at final concentrations of 1 and 10 ppm, directly after the membranes with conidia had been placed into the flasks (Fig. 1).

The observe the effect of ethylene on nutrient-deficient conidia of *B. cinerea*, the membrane filter was leached for 24 h on waterirrigated sterile sand (Bristov and Lockwood, 1975). The flow rate of the water (200 ml/h) during irrigation of the sand was controlled with a peristaltic pump.

In some experiments 15 g samples of soil in serum flasks were incubated for 1 to 5 days with either 1 or 10 ppm ethylene before the conidia were added. In other experiments, PDA slant cultures of *B. cinerea* were treated with 1 or 10 ppm ethylene for 3 days before conidia were collected for germinations tests. All counts were made in six-fold and the experiments repeated at least twice. The results were subjected to Wilcoxon's two sample test.

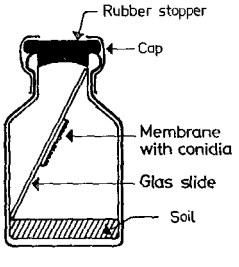

Fig. 1. Assembled serum flask with glass-slide supporting a membrane filter with conidia and containing 15 g of soil or sterile pure sand.

Fig. 1. Serumflesjes met daarin een objectglaasje waarop een membraan filter met conidiën is gemonteerd. Het flesje bevat bovendien 15 g grond of 15 g steriel zuiver zand.

Estimation of ethylene. Ethylene concentrations in serum flasks were estimated by taking 1.0 ml samples from the flasks and injecting these into a Becker multigraph 409.

Results

Inhibition of conidial germination by volatiles from soil. Inhibition of *B. cinerea* conidia above KA, F, and WW soils could already be estimated after 5 h (Fig. 2). When membranes with inhibited conidia were transferred to PDA, the percentage of germination increased to 85–95% within 24 h, demonstrating that the volatile inhibitor is fungistatic rather than fungicidal.

The responses to the atmosphere above soil samples of different origin, pH, and organic content are presented in Table 1. Germination of *B. cinerea* conidia was significantly inhibited after 24 h above all the soils tested, except Flevo 2 and 3. *C. sativus* conidia were significantly inhibited above all the soils except Lienden and Mull. In most cases the germination of *Cochliobolus* conidia was less affected than the germination of conidia of *Botrytis*, possibly because the conidia of *Cochliobolus* are less dependent on external nutrients for germination (Steiner and Lockwood, 1969).

Emanations from both alkaline and acid soils caused strong inhibition of germination. The addition of chitin or lime to Lienden soil suppressed the germination of the two fungi to a higher degree. The percentage inhibition is not correlated with organic matter content of soils.

Production and absorption of ethylene by soils. Ethylene could not be detected after 15 g of any of the alkaline soils tested were incubated in serum flasks for 24 h (Table 2). In two successive experiments the air above Lienden soil with 1% chitin contained only 6.0 nl (0.2 ppm) and 4.0 (0.13 ppm) ethylene respectively. Most of the soils had

Fig. 2. Germination of *Botrytis cinerea* conidia on membrane filters in serum flasks above Flevo soil (F_1), organic (WW) and inorganic (KA) fertilized soil, and above sterile pure sand (control).

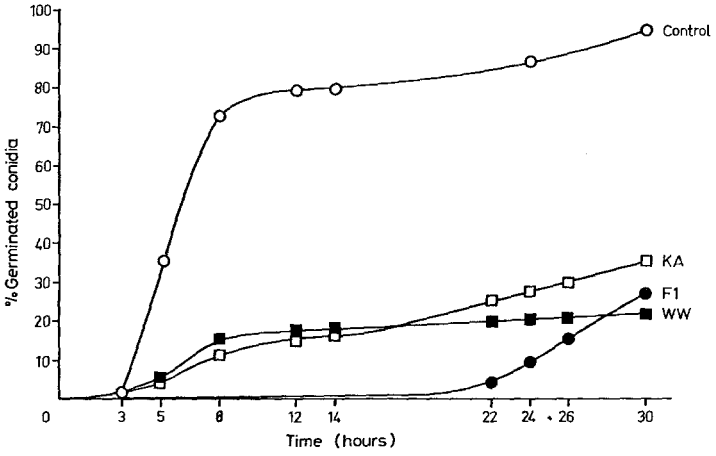

Fig. 2. Kieming van Botrytis cinerea conidiën op membraan-filters in serumflesjes boven Flevogrond (F_1), organisch (WW) en anorganisch (KA) bemeste grond, en boven steriel zuiver zand (controle).

Table 2. Production and absorption of ethylene by 15 g soil samples in 37 ml serum flasks after 24h incubation.

Soil type[1] and additions	C_2H_4 released in nl[2]	C_2H_4 absorption in nl[3]
Lienden	0	37
Lienden + chitin	6.0	5
Lienden + lime	0.8	0
Flevo 1	0	18
Flevo 2	0	37
Flevo 3	0	37
Flevo 4	0	37
Gerendal	0	10
Mull	1.5	37
Mor	2.8	30

[1] For pH and organic content see Table 1.
[2] 30 nl C_2H_4 equals 1 ppm.
[3] 37 nl C_2H_4 were injected before incubation.

Tabel 2. Produktie en absorptie van ethyleen door grondmonsters van 15 g in serumflesjes van 37 ml na 24 uur incubatie.

absorbed the injected 37 nl C_2H_4 after 24 h. Little of the C_2H_4 injected into the flasks was absorbed by Lienden soil amended with lime or chitin.

Effect of ethylene on germination of B. cinerea conidia. Germination of *B. cinerea* conidia in flasks with (Fig. 3) and without soil (Fig. 4) was not affected by ethylene.

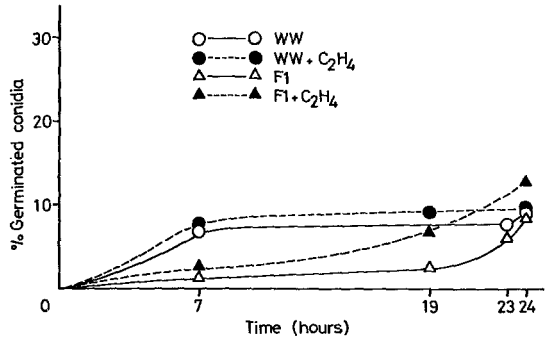

Fig. 3. Effect of 10 ppm ethylene on germination of *Botrytis cinerea* conidia above clay loam soils WW and F_1.

Fig. 3. Effect van 10 ppm ethyleen op de kieming van Botrytis cinerea conidiën boven de klei leem gronden WW en F_1.

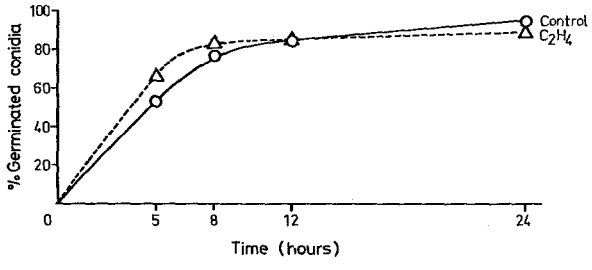

Fig. 4. Effect op 10 ppm ethylene on germination of *Botrytis cinerea* conidia.

Fig. 4. Effect van 10 ppm ethyleen op de kieming van Botrytis cinerea conidiën.

A similarly germination was not reduced if *B. cinerea* cultures had been treated with 5–10 ppm C_2H_4 for 1 to 3 days prior to the germination tests. A pretreatment of Lienden soil with either 1 or 10 ppm C_2H_4 for 3, 4 or 5 days prior to germination tests had no effect either. Leaching of the conidia did not affect their sensitivity to ethylene.

Discussion

Volatile factors inhibiting conidium germination of *B. cinerea* and *C. sativus* on membrane filters, were shown to emanate from six alkaline and two acid soils. Conidium germination was already significantly inhibited after 5 h.

Production of ethylene could not be detected in eight soils of different origin, pH and organic content that did produce fungistatic volatile factors. The addition of lime or chitin significantly increased the inhibitory action, but only chitin caused some ethylene production (0.2 ppm at most). The inhibited conidium germination above chitin-amended soil has earlier been ascribed to emanation of ammonia (Schippers and Palm, 1973).

One to 10 ppm ethylene had neither a direct effect on the conidia of *B. cinerea* nor an indirect effect in the presence of soil, even if the soil or the conidia had been pretreated with ethylene or if the spores had been deprived of nutrients by leaching. These data exclude ethylene as a factor directly or indirectly responsible for the inhibition of conidium germination of *B. cinerea* and *C. sativus* above the different soils tested and do not support the hypothesis that ethylene has a key-function in soil mycostasis (Smith, 1973, 1976).

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Samenvatting

Ethyleen niet verantwoordelijk voor kiemremming van conidiën door vluchtige stoffen uit grond

Alkalische en zure gronden van verschillende herkomst bleken vluchtige stof(fen) af te geven die de kieming van conidiën van *Botrytis cinerea* en *Cochliobolus sativus (Helminthosporium sativum)* op membraan-filters remt (Tabel 1, Fig. 2). Door toedienen van chitine of van kalk aan grond werd deze remming versterkt (Tabel 1). Ethyleen, dat door Smith (1973, 1976) in een concentratie van ± 1 ppm als een belangrijke, in grond actieve mycostatische stof wordt beschouwd, kon gas-chromatografisch niet in de mycostatische atmosfeer boven de getoetste gronden worden gedetecteerd (Tabel 2). Ook bleek de kieming van *B. cinerea* op membraan-filters in afwezigheid van grond en in aanwezigheid van grond maar zonder contact daarmee te maken, niet te worden geremd in lucht verrijkt met 1 of 10 ppm ethyleen (Fig. 3 en 4). Deze waarnemingen sluiten ethyleen uit als de verantwoordelijke stof voor

de boven de getoetste grond geconstateerde remming van de sporekieming. Zij zijn tevens in strijd met de hypotese dat ethyleen een belangrijke rol speelt in de bodemmycostasis (Smith, 1973, 1976).

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