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The influence of pH and temperature on ethylene production by mycorrhizal fungi of pine

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Abstract. The production of ethylene by six mycorrhizal fungi of pine (*Pinus sylvestris* L.) grown in media with and without methionine at temperatures of 20° C and 26° C and at pH 4.0, 6.0 or 7.0 was studied. The fungi produced more ethylene at 26° C than at 20° C, and more ethylene in media containing methionine than in media without this precursor. The fungi studied synthesized the highest amounts of ethylene at 26° C and pH 6.0.

Key words: Ethylene – Mycorrhizal fungi – pH – Temperature

Introduction

The plant growth hormone ethylene affects not only plant growth but also various biochemical processes (Ridge 1973; Jackson and Campbell 1975; Graham and Linderman 1980). Synthesis of this plant hormone has also been reported with many kinds of fungi and bacteria, both saprophytic and pathogenic. Soil bacteria produce much lower amounts of ethylene than soil fungi (Ilag and Curtis 1968; Sequeira 1973; Lynch 1975). Although methionine was found to be a precursor of ethylene, some microorganisms are able to produce this hormone in the presence of other compounds, e.g. *Penicillium* sp. isolated from soil produced ethylene in medium containing phenolic acids as the source of carbon (Considine and Patching 1975). In general, infected plants produce larger amounts of ethylene than healthy ones. Bonn et al. (1975) found considerable amounts of ethylene in plant tissues infected by *Pseudomonas solanacearum*, as well as in cultures of this microorganism. Leaves infected by *Puccinia graminis* produced more ethylene than in the uninfected ones.

Data on the production of ethylene by mycorrhizal fungi are scarce. Gogala (1991) pointed out that ethylene production by ectomycorrhizal fungi has been investigated only recently, although it is assumed that ethylene, in addition to auxins and cytokinins, is important for mycorrhizae formation and function (Slankis 1974; Gogala 1991). Because the levels of hormons produced by microorganisms are influenced by environmental factors (Strzelczyk et al. 1992; Dahm et al. 1993; Strzelczyk et al. 1994), it was thought desirable to study the effect of pH and temperature on ethylene production by ectomycorrhizal fungi.

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Materials and methods

The following fungi were studied: *Suillus bovinus*, *Hebeloma crustuliniforme* 5397, *H. crustuliniforme* 5392, *H. mesophaeum*, an unidentified ectoendomycorrhizal fungus and *Cenococcum graniforme*. The origins of the fungi studied are presented in Table 1.

The fungi were grown in Lamb's medium (Lamb 1974) in 100-ml bottles containing 50 ml of medium with or without methionine at a concentration of 200 mg/l. The experiments were carried out in duplicate. Each bottle was inoculated with a disc 10 mm in diameter cut from a 7- to 10-day-old culture fungal grown on potato dextrose agar (Difco). The fungi were incubated at 20° C or 26° C for 21 days. The pH of the media was adjusted to 5.0, 6.0 or 7.0. After 21 days of growth, 1-ml ethylene-in-air samples were withdrawn from the flasks and analysed using a gas chromatograph GCHF 18.3-4 "Chromatron" with a 100 × 0.4 cm glass column filled with silica gel (100–150 mesh) operated isothermally at 70° C with nitrogen as carrier gas and flame ionization detection ($t^0 - 140$). Pure ethylene was used as the standard for identification of this metabolite.

Results

The results obtained are presented in Tables 2, 3. It was found that *H. crustuliniforme* 5392 and *C. graniforme* grown at pH 4.0 produced more ethylene in the absence of methionine than in its presence. At 26° C, almost all fungi produced more ethylene in media with methionine. It appears from Table 2 that the fungi when grown at pH 6.0 produced considerably higher

Table 1. Isolates of mycorrhizal fungi (*ECM* ectomycorrhiza, *EEM* ectoendomycorrhiza, *X* unidentified species)

Fungal species	Isolate no.	Year of isolation	Isolated from	Source	Mycorrhiza formation	
					ECM	EEM
Suillus bovinus (L. ex Fr.) O. Kuntze	1941	1970	Sporocarp	Notec Forest, Poland	+	–
Hebeloma crustuliniforme (Bull. ex Fr.) Quel.	5397	1984	Sporocarp	Innsbruck, Austria	Not tested	
Hebeloma mesophaeum (Pers. ex Fr.) Quel.	3037	1971	Sporocarp	Notec Forest, Poland	+	–
Hebeloma crustuliniforme (Bull. ex Fr.) Quel.	5392	1974	Sporocarp	Nancy, France	+	–
EEM fungus X	Mgr X	1984	Ectoendomycorrhiza of *Pinus sylvestris*	Sekocin, Poland	–	+
Cenococcum graniforme (Sow) Ferd. et Winge	3543	1971	Ectomycorrhiza of *Abies alba*	Saint Cross Mountains, Poland	+	–

Table 2. Production of ethylene (μM/g dry mass) by mycorrhizal fungi in different culture conditions at 26° C

Fungal species	pH 4.0		pH 6.0		pH 7.0	
	Without methionine	With methionine	Without methionine	With methionine	Without methionine	With methionine
Suillus bovinus	0.0593	0.1767	0.0765	0.6500	0.0028	0.0179
Hebeloma crustuliniforme 5397	0.0025	0.0063	0.0465	0.1002	0.0070	0.0213
Hebeloma mesophaeum	0.0082	0.0101	0.5875	0.5054	0.0015	0.0172
Hebeloma crustuliniforme 5392	0.0027	0.0099	0.0247	0.1419	0.0204	0.0032
EEM fungus X	0.0018	0.0081	0.0510	0.4572	0.0072	0.0035
Cenococcum graniforme	0.0030	0.0005	0.3272	2.7250	0.0036	0.2451

Table 3. Production of ethylene (μM/g dry mass) by mycorrhizal fungi in different culture conditions at 20° C

Fungal species	pH 4.0		pH 6.0		pH 7.0	
	Without methionine	With methionine	Without methionine	With methionine	Without methionine	With methionine
Suillus bovinus	0.0037	0.0057	0.0182	0.0610	0.0065	0.0044
Hebeloma crustuliniforme 5397	0.1120	0.0286	0.0123	0.0078	0.0032	0.0025
Hebeloma mesophaeum	0.0250	0.0011	0.0167	0.0167	0.0013	0.0023
Hebeloma crustuliniforme 5392	0.0480	0.0117	0.0016	0.0448	0.0067	0.0037
EEM fungus X	0.0608	0.0187	0.0011	0.0225	0.0029	0.0015
Cenococcum graniforme	0.0052	0	0.0172	0.0112	0	0.031

amounts of ethylene in the presence of methionine than in media lacking methionine, the amounts ranging from 0.1002 to 2.7250 μM/g dry mass.

The results in Table 3 show that the fungi grown at 20° C produced less ethylene than those grown at 26° C. *S. bovinus* produced ethylene at all pH values tested, but most at pH 6.0. Both isolates of *H. crustuliniforme* cultured with methionine at pH 4.0 or 7.0 produced more of this plant growth hormone than in the absence of its precursor. Methionine added to the medium at pH 6.0 strongly stimulated ethylene production by *H. crustuliniforme* 5392, but slightly inhibited it in the case of isolate 5397 (Table 3). Similarly the ectendomycorrhizae-forming fungus formed about 20 times more ethylene with than without methionine at pH 6.0. In *C. graniforme* no ethylene production was recorded either in media with methionine at pH 4.0 or without this precursor at pH 7.0, despite good growth of the fungus. Methionine stimulated the production of ethylene by *C. graniforme* grown at pH 7.0.

Discussion

Ethylene is an endogenous plant growth regulator. It is produced not only by plants but also by soil microorganisms, both pathogenic and saprophytic (Axelrood-McCarthy and Linderman 1981; Michniewicz et al. 1983; Kampert et al. 1989; Strzelczyk et al. 1989; Gogala 1991).

Ethylene appears to be involved in plant pathology in three ways: it may provide a stimulus to resistance, it may induce symptoms of disease, and it may cause natural resistance in plants (Pegg 1976). Production of this substance may also be of ecological significance. Thus the study of the role of this plant hormone in host-parasite relationships appears worthwhile.

It is important to know the effect of chemical and physical factors on production of ethylene by microorganisms. Mycorrhizal fungi are of special interest because ethylene production by ectomycorrhizal fungi has been investigated only recently (Gogala 1991). The levels of hormones produced by microorganisms may be influenced by different ecological factors, of which pH and temperature deserve special attention.

The pH of the rhizosphere is an obvious factor influencing microbial activity, and as the rhizosphere pH may differ from the pH of adjacent root free soil by 1–2 units, this may have a significant effect on microorganisms (Curl and Truelove 1986).

Temperature is a further biologically significant variable in the soil. Both rhizosphere and bulk-soil microorganisms are exposed to a wide range of temperatures depending upon plant cover, soil moisture and soil depth (Griffin 1972). Light is also a factor affecting ethylene production; lack of light diminishes the production of ethylene by *E. coli* (Primose 1976). Methionine and other substances (phenolic acids, yeast extract, glucose) influence the production of ethylene by bacteria and fungi (Primose 1976).

The amount of ethylene produced also depends on the age of the culture. It was found that *Pseudomonas solanacearum* grown in potato-dextrose and in mineral media produced the highest amounts of this substance after 10 days of culture (Sequeira 1973). *Trichoderma viride* was found to produce more of this gas in 21- than in 7-day-old cultures (Strzelczyk et al. 1989). Decomposition of ethylene may occur in old cultures. Mycorrhizal fungi produced ethylene in media supplemented with methionine (Graham and Linderman 1980). In our studies pH distinctly affected ethylene production by the ectomycorrhizal fungi studied. The fungi grown at 26° C in general produced more ethylene in media with than without methionine (especially at pH 6.0). Considerable differences in ethylene production were found among the fungi studied.

Methionine is reported by many workers to be the precursor of ethylene, but this amino acid is not a common constituent of root exudates of most plants. It was detected in the exudates of peas, rice, wheat, clover and Monterey pine (Rovira 1965). Other soil substances essential or stimulatory for microbial production of ethylene may exist.

The effect of auxins on mycorrhizae formation and function has received much attention. This, however, cannot be said about ethylene. Therefore, it seems that further studies of the effect of this growth regulator on mycorrhizal fungus host-plant relationships are required.

Studies on the production of ethylene by mycorrhizal fungi in the presence of root exudate compounds (amino acids or organic acids) are in progress in our laboratory, and production of this gas in the presence of phenolic compounds is also being investigated.

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