Antibiotics in Sphagnum Moss

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Horticulturists have used sphagnum moss as a seed germination medium for years. It has a high moisture-holding capacity and provides good aeration. In addition to the favorable physical features, seedlings germinated in sphagnum moss have a lower incidence of "damping off," a term used to describe the rapid death of seedlings caused by several fungi including *Pythium, Rhizoctonia,* and *Fusarium* spp. Substances have been extracted from sphagnum which inhibit the growth of fungi associated with damping off. One source of the fungistatic substances is bacteria in the genus *Pseudomonas* which grow in association with sphagnum moss. The species of *Pseudomonas* producing inhibitory substances varied in sphagnum collected from different geographical areas. One of the substances is tropolone.

INTRODUCTION

Understanding natural biological control systems which have evolved over long periods of time may provide information basic to environmentally friendly pest management systems of the future. Many synthetic substances used for the control of fungi have or are in the process of being removed from commercial use because of environmental or human health concerns. Knowledge from studying natural biological control systems can help in the development of alternative pest management strategies which would contribute to the long range improvement and sustainability of U.S. agriculture.

Horticulturists have used shredded or screened sphagnum moss as a germination medium for years. It may be used alone or as a layer over another germination medium. Sphagnum moss has a high moisture-holding capacity because the leaves and stems are comprised of groups of large water-holding cells. It also provides good aeration. However, the primary reason for its use is that seedlings germinated in sphagnum moss have a much lower incidence of "damping off," a term used to describe the rapid death of young seedlings caused by a number of phytopathogenic fungi in the genera *Pythium, Rhizoctonia,* and *Fusarium*. Hope et al. (1941) measured germination percentages on a wide range of plant material with and without the presence of sphagnum moss. In all cases, the use of sphagnum moss enhanced the percentage germination and survival of the seedlings. They also showed that live sphagnum moss and dried sphagnum moss were equally effective in preventing damping off.

Sphagnum moss should not be confused with sphagnum peat, the decomposed residue produced from moss in bogs. Sphagnum peat also may be suppressive to diseases caused by soil-borne plant pathogens. The suppressiveness to damping off and root rots caused by *Pythium ultimum* is related to the decomposition level of the peat (Boehm and Hoitink, 1992). The least decomposed, light peat has the greatest suppressiveness.

Fleming and Hess (1964) reported that a substance could be extracted from sphagnum moss which inhibited the growth of the damping off organisms, *Pythium, Rhizoctonia,* and *Fusarium.* They also suggested that a source of the inhibitory substance may be a bacterium growing in association with sphagnum moss. Granhall and Hofsten (1976) have shown through electron microscopic studies that intracellular organisms such as blue green algae and bacteria are found in the large hyaline cells of sphagnum. Many bacteria were found embedded in the mucilaginous material lining the insides of the hyaline cells. Dal Vesco (1974-75) observed that samples from sphagnum-dominated vegetation were poor in fungal species diversity. He suggested that the inhibiting properties of sphagnum moss make plant residues less degradable.

Hess and Hess (1982) isolated two fungistatic substances from sphagnum moss and from pure cultures of sphagnum-associated bacteria grown in potato dextrose broth. Preliminary thin layer chromatography and chromogenic reagent data indicated that the fungistatic substances may be phenolic acids.

Thus, a number of researchers over a long period of time has demonstrated or observed the presence of inhibitory substances associated with sphagnum moss. On the basis of the literature and our own research, there clearly are substances in sphagnum moss which inhibit the growth of other organisms.

CURRENT RESEARCH

In the current research, scanning electron microscopy has shown a high population of bacteria on the surface of freshly harvested sphagnum shoots and electron microscopy confirmed the presence of bacteria embedded in the mucilaginous material lining the insides of the hyaline cells. The bacteria producing the fungistatic substances are gram negative and rod shaped with polar flagella. Based on metabolism on differential media, the genus of the bacteria is *Pseudomonas.* The species of the bacteria differed depending on where the sphagnum moss was collected.

There are three fungistatic inhibitors found in extracts of sphagnum moss or bacterial cultures. The R_f values of the inhibitors from sphagnum and bacterial cultures are the same in thin layer chromatography (TLC). The R_f values on a silica gel plate using a benzene : methanol : glacial acetic acid (180 : 32 : 16, by volume) solvent system were 0.2, 0.4, and 0.7. Attempts to identify the inhibitor at R_f 0.7 were made because it was present in the largest amounts in extracts from sphagnum moss and bacterial cultures. This inhibitor reacted to chromogenic reagents as follows: diazotised sulfanilic acid - orange red to red; ferric chloride/ferric cyanide - blue; and bromocresol green - yellow. The reactions with the first two reagents indicated the presence of a phenolic functional group and the bromocresol green reaction indicated a carboxylic acid group.

Bacteria isolated from sphagnum moss collected in Millston, Wisconsin produced large quantities of the inhibitor found at R_f 0.7. A sufficient quantity of the inhibitory substance was extracted to permit crystallization. X-ray crystallography was used to identify the inhibitor as tropolone. Tropolone is a non-benzenoid, aromatic compound with a seven-membered ring. It is a weakly acidic compound with a hydroxyl group at position seven and a carboxyl group at position one accounting for the phenolic-acid-like reactions to the chromogenic reagents described above.

One of the early reports of the antibiotic activity of tropolone was made by Pauson (1955) who investigated why western red cedar (*Thuja plicata*) was so resistant to decay and attributed the presence of a derivative of tropolone to its durability. Lindberg et al. (1980) and Lindberg (1981) reported the isolation of a tropolone producing *Pseudomonas* sp. from Bermuda grass (*Cynodon dactylon*) and demonstrated that tropolone was toxic to *Pythium* and other fungi.

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