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Interactions between the mycoparasite *Pythium oligandrum* and sclerotia of the plant pathogen *Sclerotinia sclerotiorum*

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Abstract

Pythium oligandrum Drechsler is a mycoparasite which parasitizes hyphae of many fungal species. A detailed study of the interactions between *P. oligandrum* and the sclerotia of the plant pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary is presented. *Pythium oligandrum* was present in Danish soils at concentrations between 4 and 26 cfu g^{-1} soil. An increase in the natural population of *P. oligandrum* by addition of *P. oligandrum* zoospores to a soil reduced the ability of sclerotia of *S. sclerotiorum* to germinate myceliogenically and the sclerotia were colonized internally by *P. oligandrum*. This colonization and reduction of germination of sclerotia were also seen when sclerotia and *P. oligandrum* were incubated together in water. Small sclerotia were significantly more susceptible to parasitism by *P. oligandrum* than large sclerotia, and increasing the incubation time caused a further reduction in the germination ability of the sclerotia. *P. oligandrum* was able to pass through its entire life-cycle from zoospores to oogonia both with sclerotia as sole nutrient-source and in water containing exudates from the sclerotia. The cell wall degrading enzymes N-acetyl-β-D-glucosaminidase (NAGase), endo-chitinase, protease, β-glucanase, β-glucosidase and cellobiohydrolase were detected in culture filtrates of *P. oligandrum* cultivated with *S. sclerotiorum*. These findings suggest that *P. oligandrum* has a potential to reduce the survival of *S. sclerotiorum* sclerotia present naturally in soils, through mycoparasitic activity.

Introduction

Pythium oligandrum Drechsler is a potential biocontrol agent. Based on hyphal–hyphal interactions it has a wide host range (Deacon, 1976; Laing and Deacon, 1991; Ribeiro and Butler, 1995), but whether it can parasitize the resting structures of the same host species is not known. Several studies have described the parasitic ability of *P. oligandrum* on hyphae of different host fungi when grown on an agar medium (e.g. Deacon, 1976; Lewis et al., 1989; Bradshaw-Smith et al., 1991; Laing and Deacon, 1991). However, the mycoparasitic ability of *P. oligandrum* in soil, has not been investigated in detail.

During mycoparasitism, *P. oligandrum* receives nutrients from the host resulting in the production of numerous oospores (Deacon and Henry, 1978). This growth of the mycoparasite at the expense of the host may be one of the mechanisms by which *P. oligandrum* in some investigations has protected crop plants against seedling diseases under experimental conditions (Vesely and Hejdanek, 1984; Lutchmeah and Cooke, 1985; McQuilken et al., 1990). On the other hand, low levels of disease control have also been observed (Gordon-Lenox et al., 1987; Walther and Gindrat, 1987). The poor disease control may be related to the use of oospores as soil inoculum, since oospores germinate slowly and poorly in soil (Walther

and Gindrat, 1987; McQuilken et al., 1990). Encysted zoospores have been suggested as an alternative soil inoculum, as they can initiate mycoparasitism, establish in the soil (Madsen et al., 1995) and germinate quickly in soil (Madsen, 1996).

Pythium oligandrum has been isolated from sclerotia of *S. sclerotiorum* which had been placed on soil (Ribeiro and Butler, 1992), but studies of the effect of *P. oligandrum* on the sclerotia have not been published. Sclerotia of *S. sclerotiorum* are reported to survive at least two to five years in soil (Williams and Western, 1965; Adams and Ayers, 1979) and approximately 90% of the life-cycle is spent in soil as sclerotia (Adams and Ayers, 1979); secondary sclerotia can also form from decaying sclerotia (Coley-Smith and Cooke, 1971; Huang, 1980). The aim of this research was to study the effect of *P. oligandrum* on *S. sclerotiorum* sclerotia. As a basis for these studies, the presence of *P. oligandrum* in seven Danish soils was studied; interactions between *P. oligandrum* and sclerotia of *S. sclerotiorum* were then investigated using *P. oligandrum*. The internal colonization of sclerotia by *P. oligandrum* was studied, and the survival of the sclerotia was estimated. In order to determine relationships involved in the interactions between the mycoparasite and sclerotia, the following factors were investigated: the ability of *P. oligandrum* to utilize nutrients released by the sclerotia; the production of hydrolytic enzymes, and the ability of *P. oligandrum* to utilize carbohydrates present in sclerotia.

Material and methods

Fungal material

The pathogen *Sclerotinia sclerotiorum* (isolate CP1517) was isolated from carnations in Denmark by E. Hellmers. The mycoparasite *Pythium oligandrum* Drechsler (isolate MM1) was isolated from a Danish soil and selected because of its relatively high zoospore production. In one of the soil studies, a metalaxyl-tolerant mutant (developed by Madsen et al., 1995) of this isolate was used. Sclerotia of *S. sclerotiorum* were produced on potato-dextrose agar (PDA, Difco Labs, Detroit, USA) at 20 °C in 22–28 day old cultures. Zoosporangia of *P. oligandrum* were produced on V8-juice-agar (200 ml V8-juice, Campbell Soup Company, USA; 800 ml water; 15 g Difco Bacto agar; 3 g $CaCO_3$) placed in water and zoospores released after treatment with cold water, as described in Madsen et al., (1995). In a single study, *Fusarium culmorum* (W.G. Sm.) Sacc. grown on PDA was used as a host for *P. oligandrum*.

Detection of P. oligandrum *in Danish soils*

After harvest, seven soils (clay-loams and sandy-loams) were collected from horticultural and agricultural fields from Zealand, Denmark. Each sample was bulked from ten sub-samples from the top 10 cm of the profile, then soils were air-dried and sieved (2.0 mm mesh) and finally stored in polyethylene bags at +5 °C for one to six weeks. Soil pH was between 6.0 and 7.2 (measured in a soil–water mixture 2 : 1 (v/v)).

F. culmorum-colonized agar was used for selective detection of *P. oligandrum* in the soils (Madsen et al., 1995; Mulligan et al., 1995). For this, PDA plates were colonized by *F. culmorum*, then each plate was cut into six sectors which were placed in separate Petri dishes. The soil was diluted with dried sterile sand up to a dilution of one part soil in 32 parts total, and mixed with a vortex mixer. A total of 0.4 g air-dried diluted or non-diluted soil was placed on part of each sector and twelve sectors were used for each soil and dilution level. A volume of 100 µl water was added to the 0.4 g soil. The Petri dishes were incubated in darkness at 25 °C. The sectors were examined microscopically every third day for presence of *P. oligandrum* oogonia until day 21. Number of infective *P. oligandrum* units g^{-1} soil was estimated by most probable number (MPN) analysis (Fisher and Yates, 1963).

Ability of P. oligandrum *to colonize sclerotia and to inhibit their germination in unsterile soil or water*

Zoospores (6.25×10^3 spores g^{-1} dry soil) of the metalaxyl-tolerant mutant of *P. oligandrum* were inoculated to a natural (unsterile) soil (a Danish agricultural soil pH 6.4, sandy-loam, where *P. oligandrum* was naturally present at 6 cfu g^{-1}). A total of 88 *S. sclerotiorum* sclerotia were placed in a gauze bag (mesh size 0.8 mm) on 15 g water saturated soil in a glass Petri dish and covered with another 5 g of water saturated soil. After five days of incubation at 18 °C, the sclerotia were removed and placed on P_5ARPM (P_5ARP is a *Pythium* and *Phytophthora* selective medium according to Jeffers and Martin (1986), here modified by adding 40 µg ml^{-1} metalaxyl) in an attempt to detect the metalaxyl-tolerant *P. oligandrum* on the sclerotia.

In another experiment, sclerotia were classified according to length: (A) 1.5–2.5 mm; (B) 3–5 mm and (C) 6–15 mm. A total of 250 sclerotia of each size were placed in gauze bags (125 sclerotia in each bag and Petri dish) in soil treated as above, with or without *P. oligandrum* zoospores. The Petri dishes containing the soil and fungi were incubated at 18 °C for five days. To examine if the sclerotia were able to germinate and if they were colonized by *P. oligandrum* after the incubation, the sclerotia were washed and surface sterilized in 96% ethanol and 5% Na-hypochlorite for 30 s, before they were placed on P_5ARP or on PDA_{Cl} (Cl = chloramphenicol (0.1 g l^{-1} PDA) which inhibits growth of *P. oligandrum*). The germination ability of the sclerotia was estimated four days after they were placed on PDA_{Cl}, and likewise the examination for the growth of *P. oligandrum* was made on day 4. The experiment was carried out three times. The soil was stored dry at room temperature for a month between each experiment, hence the three repetitions are called experiment 1, 2 and 3. Data were binomially distributed and analyzed by Odds ratio analysis (see e.g. Jørgensen et al., 1998) in the GENMOD procedure in SAS. To study the effect of longer incubation time, a total of 250 sclerotia of each size were treated as above, but observed after an incubation period of twelve days. The experiment was carried out twice.

The ability of *P. oligandrum* zoospores to colonize and kill sclerotia in water was investigated. Sixty sclerotia of each of the three sizes (A, B, C) were placed in a 10 ml zoospore suspension (10^4 ml^{-1}), and incubated at 18 °C for five days. The internal colonization and survival were tested as above. The experiment was repeated twice.

Physiology in relation to utilization of sclerotia

Sclerotia (0.3 g) were placed in a water suspension (10 ml) of zoospores (2.4×10^3 ml^{-1}) in Erlenmeyer flasks (150 ml), or in water without zoospores. Furthermore, zoospores in water were incubated separately in Erlenmeyer flasks. The temperature was 20 °C. Test samples of 0.3–0.6 ml were collected at different times. The test samples were centrifuged for 10 min at 11,000*g* and supernatants were used for the enzyme-assays. The experiment was carried out three times.

CM-cellulose–RBB (Remazol Brilliant Blue) was used as the substrate for the assay of endo-cellulase (endo-1,4(1,3)-β-D-glucanase) (EC 3.2.1.4) activity; CM-Curdlan–RBB for the assay of β-glucanase (endo-1,3(1,4)-β-glucanase) (EC 3.2.1.6) activity; CM-chitin–Remazol Brilliant Violet for the assay of endo-chitinase (EC 3.2.1.14) activity and Gelatin–RBB as a substrate for protease activity (i.e trypsin EC 3.4.21.4). All enzyme substrates were supplied by Loewe Biochimica Blue Substrates, Göttingen, Germany. Dye-labelled substrate in aqueous solution (100 µl), culture filtrate supernatants (150 µl) and 0.1 M phosphate buffer (KH_2PO_4, Na_2HPO_4), (150 µl) were mixed in Eppendorf tubes and incubated for 80 min at 37 °C. The pH values of the buffers used were 6.0, 5.0, 5.0 and 7.0 for CM-cellulose–RBB, CM-Curdlan–RBB, CM-chitin–RBB and Gelatin–RBB respectively, and the reactions were terminated by the addition of 250, 250, 200 and 300 µl 2 N HCl respectively. Subsequently, the tubes were cooled at 0 °C for 15 min and centrifuged (5 min at 11,000*g*). Appropriate controls without the enzyme were run simultaneously. Supernatants (200 µl) containing dye-labelled degradation products were transferred to micro-titer plates (96 wells, 350 µl cavities Framonlast, France), and measured spectrophotometrically (Ceres UV 900 HDI, Bio-tek, Instruments, Inc, Winooski, VT, USA) at 595 nm for substrates labelled with RBB. Endo-chitinase activity was assayed at 550 nm. The activity of the individual enzyme was expressed as a percentage of the highest level of activity obtained for that enzyme in all three kinds of fungal cultivations (i.e zoospores and sclerotia; zoospores alone; sclerotia alone).

For the assay of exo-acting enzymes, the methods of Claeyssens and Aerts (1992) were used. To quantify the activities of N-acetyl-β-D-glucosaminidase (EC 3.2.1.30) (designated NAGase) the release of *p*-nitrophenol from *p*-nitrophenyl-N-acetyl-β-D-glucosaminide was estimated. To quantify the cellulytic activities of β-glucosidase (EC 3.2.1.21) and cellobiohydrolase (EC 3.2.1.91) the release of *p*-nitrophenol from *p*-nitrophenyl-β-D-glucopyranoside or *p*-nitrophenyl-β-D-celloside was estimated. All substrates were supplied by Sigma Chemical Company, USA. Culture filtrates (30 µl) were added to wells in micro-titer test plates. Fifty µl of substrate solution (4 °C) containing 300 µg ml^{-1} of *p*-nitrophenyl-N-acetyl-β-D-glucosaminide, or *p*-nitrophenyl-β-D-N,N-diacetylchitobiose, or *p*-nitrophenyl-β-D-glucopyranoside or *p*-nitrophenyl-β-D-cellobioside dissolved in 50 mM potassium phosphate (pH 6.7) was added to the wells. Appropriate controls without either the enzyme or the substrate

were run simultaneously. The plates were incubated for 5–50 min at 50 °C (NAGase activities) or 37 °C (cellulytic activities). Reactions were terminated and the yellow colour developed following the addition of 50 µl 0.4 M Na_2CO_3 to each well. Absorbance was measured at 405 nm. Activity was expressed as pkatals (pmoles of nitrophenol released $s^{-1}\,ml^{-1}$ of culture filtrate).

Protein content of the culture filtrate was determined by Bio-Rad Protein Assay with bovine serum albumin as a standard (Bio-Rad Laboratories, Richmond, CA 94804, USA), according to the directions of the manufacturer.

The ability of the *P. oligandrum* isolate to utilize different sugars present in a standard medium as sole carbon source was studied as in Foley and Deacon (1986a). The sugars were mannitol (12 g l^{-1}), glucose (12 g l^{-1}), glycerol (20 ml l^{-1}), arabitol (14 g l^{-1}) or trehalose (14 g l^{-1}) and as a control one medium without any carbohydrates was included.

Utilization of exudates from the sclerotia

Sclerotia were placed in sterile, demineralized water (120 g fresh weight l^{-1}) for 40 h at 20 °C. The water (referred to as 'sclerotium water') was decanted from the sclerotia and sterile filtered (pore size 0.2 µm, Sartorius, Minisart, Germany). A volume of 10 ml of *P. oligandrum* zoospores ($2.2 \times 10^3\,ml^{-1}$) was incubated in 10 ml 'sclerotium water'. To determine whether *P. oligandrum* zoospores were able to establish in 'sclerotium water', which contained only exudates from sclerotia, observations of growth from zoospores were recorded after seven days of incubation at 20 °C. To see whether endo-chitinase, protease and NAGase would be induced in the absence of sclerotia but in the presence of sclerotium exudates, *P. oligandrum* was cultivated in 'sclerotium water' and enzyme assays performed as described earlier.

Results

Detection of P. oligandrum *in Danish soils*

Pythium oligandrum was present in all the seven soils studied. MPN analysis showed that *P. oligandrum* was present in the soils in a concentration between 4 and 26 cfu g^{-1} soil and in a mean concentration of 15 cfu g^{-1} soil. The highest concentration was found in a clay-loam with pH 7.2.

Ability of P. oligandrum *to colonize sclerotia and to inhibit their germination in non-sterile soil or in water*

When zoospores of the metalaxyl-tolerant isolate of *P. oligandrum* were incubated in the soil with sclerotia of *S. sclerotiorum* for five days, 73% of the sclerotia were internally colonized by the metalaxyl-tolerant *P. oligandrum* isolate. The sclerotia colonized by *P. oligandrum* appeared to be soft and decomposed. Sclerotia, which survived in the zoospore-treated soil or in zoospore suspension, germinated with fewer hyphae than did the sclerotia incubated in non-treated soil or in water (data not shown).

Treatment of a soil with *S. sclerotiorum* sclerotia using *P. oligandrum* zoospores had a significant effect on the germination of the sclerotia in all three experiments (Table 1). Between 64% and 85% of sclerotia from the non-treated soil germinated, while 24–45% from the *P. oligandrum*-treated soil germinated. A significant ($P \leq 0.05$) effect of sclerotium size of the treated sclerotia was found and the germination of the large (size C) sclerotia was significantly greater than the germination of the small (size A) sclerotia. Internal colonization by *P. oligandrum* was significantly higher in size A than size C sclerotia (Table 1).

When the incubation time was extended from five to twelve days, the survival of sclerotia was reduced. Only 2.7% of the size A sclerotia from the zoospore-treated soil and only 50.5% of the sclerotia from the non-treated soil were able to germinate. The survival proportions were the same for size B and C sclerotia; 10.4% treated compared with 61.8% non-treated size B, and 20.5% treated compared with 80.0% non-treated size C. The sclerotia from the treated soil were decomposed to a degree making sterilization of the surface alone impossible.

Sclerotia incubated in water with zoospores of *P. oligandrum* sank to the bottom of the flasks, a mycelium with oospores of *P. oligandrum* developed on and between them, and the water became brown. When either of the fungi were incubated in water separately, the water remained colourless. After sixteen days incubation of sclerotia in water with $2 \times 10^4\,ml^{-1}$ zoospores, the zoospores developed to approx. $2 \times 10^6\,ml^{-1}$ oogonia, in addition to sporangia and mycelium. Most (71%) of the tested sclerotia were colonized internally by *P. oligandrum* on day 5, the sclerotial germination was clearly reduced (Table 2) and some sclerotia were clearly so

Table 1. Germination of *S. sclerotiorum* sclerotia of size[1] A, B or C five days after sclerotia were incubated in a natural soil treated with *P. oligandrum* zoospores (treated) or in a non-treated soil and internal colonization by *P. oligandrum* of sclerotia selected from treated soils

	Germination[2] (treated %)	Germination (non-treated %)	Colonization[2]
Experiment[3] 1			
Size A	40[a]	67[a]	46[a]
Size B	40[a]	80[b]	31[b]
Size C	54[b]	83[b]	29[b]
$\bar{x}$	**45[a]**	**77[b]**	35
Experiment 2			
Size A	42[a]	75[a]	35[a]
Size B	39[a]	79[ab]	39[a]
Size C	54[b]	85[b]	27[b]
$\bar{x}$	**45[a]**	**79[b]**	34
Experiment 3			
Size A	24[a]	78[a]	45[a]
Size B	25[a]	64[b]	41[ab]
Size C	38[b]	75[ab]	31[b]
$\bar{x}$	**29[a]**	**77[b]**	39

[1]Size of sclerotia: A 1.5–2.5 mm; B 3–5 mm and C 6–15 mm. About 75 sclerotia of each size, in each treatment and experiment were used for studying the sclerotial germination, while about 50 sclerotia of each size were used in each experiment for studying the colonization of sclerotia by *P. oligandrum*.
[2]The germination abilities of sclerotia of the three sizes and the colonization of sclerotia of the different sizes are both separately compared by Odds ratio analysis (PROC GENMOD). Numbers within the same column and the same experiment followed by the same letter are not significantly different at the 5% level. Within rows, bold values that are followed by the same letter are not significantly different according to a Odds ratio analysis.
[3]Experiment 1, 2 and 3 are repetitions of the same experiment, but the soil had been stored for one month between the successive experiments.

Table 2. Germination of size[1] A, B or C *S. sclerotiorum* sclerotia, after five days in water with *P. oligandrum* zoospores (treated) or in water without zoospores (non-treated) and internal colonization by *P. oligandrum* of sclerotia after treatment with zoospores

	Germination (treated %)	Germination (non-treated %)	Colonized (%)
Size A[1]	29[a]	100[a]	92[a]
Size B	58[b]	100[a]	69[a]
Size C	64[b]	100[a]	60[a]
$\bar{x}$ treated	**50[a]**	**100[b]**	71

[1]For explanation of conventions see Table 1.

decomposed that they were not assessed for germination or colonization.

Physiology in relation to utilization of sclerotia

Pythium oligandrum zoospores were not able to develop in water alone. Signs of interactions between the *P. oligandrum* zoospores and the sclerotia were observed very early, as enzyme activities were induced or inhibited as early as a few hours after cultivation of the two fungi together (Figures 1 and 2).

A NAGase activity was detected when the sclerotia were incubated alone in water. Over time, the NAGase activity increased more when the zoospores were cultivated together with the sclerotia than when the sclerotia were incubated alone (Figure 1). The NAGase produced both when sclerotia were incubated alone in water or cultivated with *P. oligandrum* exhibited activities in the investigated temperature interval (16–95 °C) and had optimal activities between 70 °C and 85 °C (data not shown). The endo-chitinase activity was detected after 24 h and was subsequently greater in the culture filtrate where the two fungi had been cultivated together than when sclerotia were incubated alone (Figure 2).

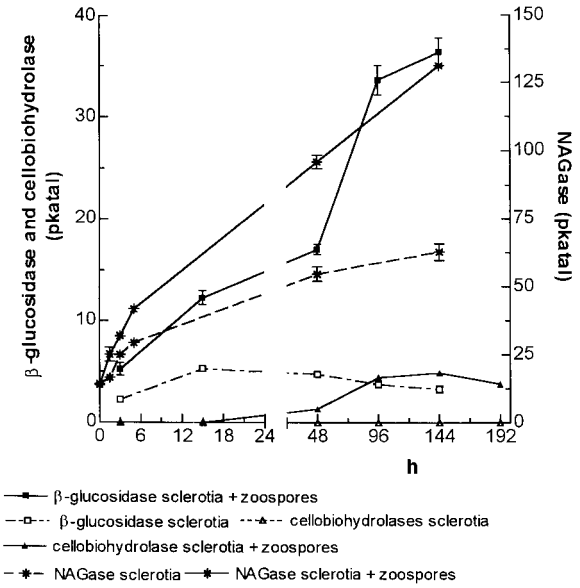

Figure 1. Activity of cellobiohydrolase and β-glucosidase and of NAGase in culture filtrates of *P. oligandrum* zoospores and *S. sclerotiorum* sclerotia or of *S. sclerotiorum* sclerotia separately in water. Mean values ± S.E.

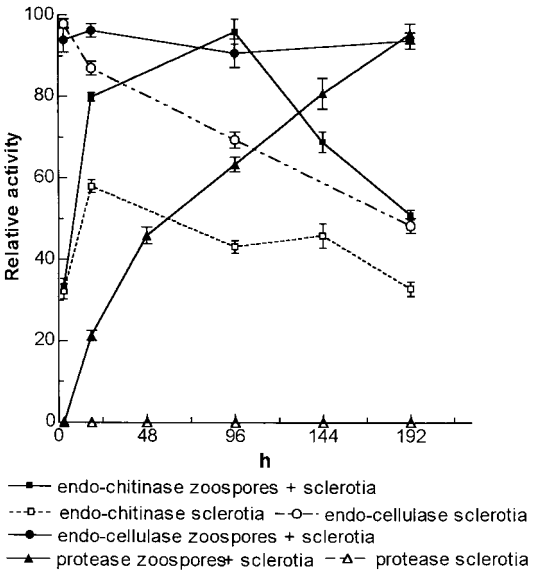

Figure 2. Activity of endo-chitinase, protease and endo-cellulase in culture filtrate of *P. oligandrum* zoospores cultivated together with *S. sclerotiorum* sclerotia in water or of *S. sclerotiorum* sclerotia alone. The enzyme activities are expressed relative to the maximum activity of each enzyme. Mean values ± S.E.

Protease activity was not detected when sclerotia were alone in water. However, when zoospores were cultivated together with sclerotia, protease activity increased (Figure 2). When sclerotia were alone in water, the protein content of the culture filtrate increased from $19.3 \pm 1.2\ \mu g\ ml^{-1}$ after 3 h to $44.2 \pm 2.0\ \mu g\ ml^{-1}$ on day 4. When sclerotia were cultivated together with zoospores the protein content of the culture filtrate increased from $17.5 \pm 0.6\ \mu g\ ml^{-1}$ (3 h) to $40 \pm 2.3\ \mu g\ ml^{-1}$ (four days).

An increasing β-glucosidase activity was detected when sclerotia and zoospores were cultivated together (Figure 1). The sclerotia alone had low β-glucosidase and endo-cellulase activities. Cellobiohydrolase was detected after 48 h, when the two fungi were incubated together (Figure 1). Activity of endo-1,4(1,3)-β-glucanase was detected when the two fungi were cultivated together (data not presented). In addition to the production of exocellulases, it was seen that *P. oligandrum* was able to utilize mannitol, glycerol, glucose, arabitol and trehalose as sole carbon source (data not presented).

Utilization of exudates from the sclerotia

P. oligandrum zoospores were able to establish in the 'sclerotium water' by encystment, germination, mycelium growth and production of oospores and zoosporangia. The detection of protease and endo-chitinase activities confirmed the establishment. On day 4 the extracellular protease, endo-chitinase and NAGase activities in culture filtrate of *P. oligandrum* zoospores in 'sclerotium water' were 37%, 58% and 36%, respectively, of the corresponding activities in culture filtrates from sclerotia and zoospores cultivated together. However, 'sclerotium water' in itself also showed endo-chitinase and NAGase activities (but no protease activity), and for endo-chitinase, the activity was 51% of the activity in culture filtrate of zoospores incubated in 'sclerotium water' (significantly less), while the difference in NAGase activities was non-significant.

Discussion

This investigation shows that *P. oligandrum* has a potential to damage sclerotia of *S. sclerotiorum*. *P. oligandrum* colonized and parasitized sclerotia in soil and reduced their survival significantly (Table 1). It was not only the specific *P. oligandrum* isolate (MM1) which was able to parasitize the sclerotia, as *P. oligandrum* was isolated from sclerotia inoculated in soils where *P. oligandrum* was only naturally present (data not shown). The observation that *P. oligandrum* was detected in all Danish soils studied correlates well with earlier investigations in other countries and continents showing that *P. oligandrum* is a very widespread fungus (e.g. Martin and Hancock, 1986; Mulligan and Deacon, 1992; Ribeiro and Butler, 1992; White et al., 1992). In this study we have used zoospores as inoculum, which seem to be a good inoculum source as the number of *P. oligandrum* propagules was increased at least by 200 fold sixteen days after zoospores were incubated with sclerotia as sole nutrient source. It is also possible to enhance the *P. oligandrum* populations by addition of appropriate plant material (Mulligan et al., 1995; Madsen, 1996) and addition of organic material to the plant growth medium may be used as a biocontrol strategy (Mulligan et al., 1995), alone or in combination with the addition of *P. oligandrum* zoospores. In addition, the distribution of *P. oligandrum* indicates a versatile adaptive capacity.

Pythium oligandrum colonized and parasitized sclerotia in water (Table 2) which may reduce the spread of the disease if a similar process takes place in nature, since sclerotia can be spread by water (Adams and Ayers, 1979; Steadman, 1979). Smaller sclerotia were more easily colonized by *P. oligandrum*, both in water and soil, and had a lower survival (Tables 1, 2). This is in accordance with Hoes and Huang (1975), who showed that small sclerotia possess a lower nutrient reserve and are more easily destroyed by soil organisms than larger sclerotia.

The sclerotia supplied *P. oligandrum* with all necessary nutrients, since by parasitizing sclerotia it was able to complete its entire life-cycle including zoospores, cysts, mycelium, sporangia and oogonia. *P. oligandrum* was also able to utilize nutrients released from sclerotia, and the production of exudates by a host which *P. oligandrum* is able to utilize as sole nutrient source has earlier been related to the susceptibility of the host (Foley and Deacon, 1986b). This stimulation of *P. oligandrum* growth in the presence of the sclerotia, but without contact, was probably due to the fact that sclerotia, according to Al-Hamdani and Cooke (1987) and Willetts and Bullock (1992), exude low amounts of carbohydrates similar to the carbohydrates present in sclerotia.

Chitin and β-glucan are present in the sclerotial cell walls as major components. Furthermore, protein bodies are the major cytoplasmic storage in mature sclerotia (Willetts and Bullock, 1992). The presence of the two chitinases and the proteases (Figures 1 and 2) in the culture filtrate shows a potential of enzymatic breakdown of sclerotial chitin and protein. In contrast to sclerotia themselves, the nutrients released from sclerotia did not induce a high NAGase activity, but they induced *P. oligandrum* to produce endo-chitinase and protease. The high NAGase activity induced when both fungi are cultivated together may mainly originate from *S. sclerotiorum*, which produced NAGase when imbibing in water. The β-glucans in sclerotia are of 1,3 and 1,6 linkages, hence the enzymes endo-1,3-β-glucanase (EC 3.2.1.39) and endo-1,6-β-glucanase (3.2.1.75) may, in addition to β-glucanase (3.2.1.6), also be involved in the interactions. The observation of exo-cellulase activities (Figure 2) of *P. oligandrum* in the absence of hosts with high cellulose content in the cell walls may be related to both the growth and starvation of *P. oligandrum* which contains cellulose in its cell walls. The activity of NAGase at temperatures as high as 90 °C is in accordance with the activity of the NAGase produced by *Trichoderma harzianum* (Lorito et al., 1994). The ability of *P. oligandrum* to parasitize sclerotia may partly be due to the production of such degradative enzymes. Furthermore it may be based on the ability of *P. oligandrum* to utilize glycerol, mannitol, arabitol, trehalose and glucose since glycerol and the sugars are major compounds of *S. sclerotiorum* sclerotia (Al-Hamdani and Cooke, 1987). It has earlier been reported that two *P. oligandrum* isolates were able to utilize these sugars (Foley and Deacon, 1986a) and another isolate of *P. oligandrum* has been shown to be able to utilize three of the sugars (McQuilken et al., 1992). Consequently the ability to utilize these sugars present in host sclerotia may be a common feature of *P. oligandrum* species.

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