ANTIFUNGAL COMPOUNDS PRODUCED BY
EPICOCCUM PURPURASCENS AGAINST
SOIL-BORNE PLANT PATHOGENIC FUNGI

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Summary—Epicoccum purpurascens inhibited colony growth of Phytophthora spp and Pythium spp in culture more than other species tested. Six antifungal compounds were detected in cultures of E. purpurascens grown in two selective media. Four of these compounds, epicorazines A and B and two compounds (X and Y) of unknown identity (designated the epicorazine fraction) were produced simultaneously at an early stage in the growth of E. purpurascens in a sucrose plus casamino acid medium but were not detected in a glucose plus (NH₄)₂HPO₄ medium. The compound flavipin was detected in both media but was preferentially produced in the latter. A third unidentified antifungal compound was detected in both media at a late growth stage.

Mycelial growth of Pythium spp was more sensitive to the epicorazine fraction than to flavipin. Phytophthora spp tended to be more sensitive to flavipin. Both were much less toxic to mycelial growth of Fusarium oxysporum f. sp. lini. Flavipin inhibited germination of zoospores of Phytophthora cinnamomi and oogonia of Pythium intermedium.

Mycelia of Phytophthora spp and Pythium spp antagonized by E. purpurascens were stunted and swollen and where opposing cultures intermingled hyphae of E. purpurascens coiled and penetrated those of the two pathogens. Cellulase and β-1,3 glucanase activities were detected in cultures of E. purpurascens grown on Phytophthora or Pythium spp hyphal wall material as the sole carbon source.

INTRODUCTION

Epicoccum purpurascens is an ubiquitous, red-pigmented fungus which survives saprophytically on senescent plant remains both aerially and in soil (Schol-Schwarz, 1959). Antagonism between E. purpurascens and a number of plant pathogenic fungi has already been observed. Parasitism of Cochliobolus sativus (Helminthosporium sativum) by E. purpurascens reduced infection of wheat as successfully as the mycoparasite Trichoderma viride (Campbell, 1956), Rhizoctonia solani was inhibited in vitro by E. purpurascens and tight coiling by the antagonist around R. solani hyphae was observed when cultures intermingled (Chand and Logan, 1984). Of saprobes isolated from barks of wych elms and peach twigs E. purpurascens was one of the most successful antagonists of Ceratocystis ulmi which is the causal organism of Dutch elm disease (Webber and Hedger, 1986) and Cytospora cincta which causes peach canker (Royse and Ries, 1978).

Pigmented antibiotics produced by E. purpurascens include epirodins A and B (Burge et al., 1976) and flavipin (3,4,5-trihydroxy-6-methyl-0-phthalaldehyde) (Bamford et al., 1961). The epirodins inhibited Bacillus megaterium (Burge et al., 1976) and Saccharomyces cerevisiae (Ikawa et al., 1978). Flavipin, which was originally isolated from cultures of Aspergillus flavipes and identified by Raistrick and Rudman (1956), inhibited spore germination in Botrytis allii but had little antibacterial activity (Raistrick and Rudman, 1956; Bamford et al., 1961).

Epithidioketopiperazine compounds designated epicorazines A and B are two non-pigmented antibiotics produced by E. purpurascens (Baute et al., 1978). These compounds inhibited growth of Staphylococcus aureus but were apparently not assayed for antifungal activity.

We have determined the mechanisms of antagonism of E. purpurascens towards a range of soil-borne plant pathogenic fungi before testing its potential as a biocontrol agent.

MATERIALS AND METHODS

E. purpurascens Ehreth ex Schlecht (= E. nigrum Link) isolate 13, was isolated from retted flax stems (Brown, 1984) and maintained on Cooke's No. 2 medium with a mineral supplement (Swinburne, 1976). Of three other isolates compared with isolate 13 for antagonistic properties one was obtained from flax stems, another one from apple leaf scars and the third from soil isolations. The three isolates of Phytophthora cinnamomi (IMI 70473, 157800 and 129910), P. citricola (IMI 136413), P. cryptogea (IMI 69664) and P. cactorum (IMI 242091) were obtained from the Commonwealth Mycological Institute, Kew. P. syringae was isolated from a naturally-infected Bramley's Seedling apple fruit. Pythium intermedium (F) was isolated from diseased roots of flax and the other Pythium spp were obtained from Dr G. Sewell, Institute of Horticultural Research, East Malling. Phytophthora spp and Pythium spp
were maintained on potato dextrose agar (PDA, Oxoid). *Fusarium oxysporum* f.sp. *lini* was maintained and sporulated on oat meal agar. Other fungi used in this study were maintained on malt agar (2%).

**Mycelial interactions between *E. purpurascens* and other fungi.**

Dual cultures of *E. purpurascens* and some plant pathogenic fungi were established on 3.5 cm apart on PDA. The inoculum consisted of discs (4 mm) cut with a cork borer from the leading edge of fungal colonies. Five replicate Petri dishes for each dual culture were incubated for at least 7 days at 20°C. Interactions between the opposing colonies were visually assessed and the types of interaction described were based on those defined by Webber and Hedger (1986).

To observe hyphal interactions microscopically sterile horticultural silver sand was sprinkled onto PDA plates. These plates were inoculated with *E. purpurascens*, *P. cinnamomi* (IMI 122910) or *P. intermedium* (F) and incubated at 20°C for 3 to 5 days. Microscope slides were cleaned with chromic acid, rinsed thoroughly with sterile distilled water, dried and autoclaved. The slides were then dipped into molten PDA (48°C) and immediately transferred onto PDA in Petri plates. The cooled slides were inoculated at one end with *E. purpurascens* and at the other end with either *P. cinnamomi* or *P. intermedium* by transferring a single grain of sand from the previously inoculated plates. The slides were incubated at 20°C until the opposing colonies met, then they were examined microscopically and interactions recorded photographically.

**Antifungal compound production and extraction.**

Two selective liquid media were used for detection of antifungal activity produced by *E. purpurascens*. One medium contained glucose, 5 g; (NH₄)₂HPO₄, 0.5 g; KH₂PO₄, 0.5 g; MgSO₄·7H₂O, 150 mg; CaCl₂, 50 mg; FeCl₃, 10 mg in 1 l distilled water (GAP). The sucrose plus casamino acid medium (SCA) contained sucrose, 5 g; KH₂PO₄, 1 g, MgSO₄·7H₂O, 0.5 g; casein hydrolysate, 4.6 g; trace element solution, 2 ml in 1 l distilled water. The stock trace element solution contained FeSO₄·7H₂O, 26 mg; ZnSO₄·7H₂O, 22 mg; CuSO₄·5H₂O, 4 mg; MnSO₄·4H₂O, 2 mg in 100 ml distilled water.

To investigate the accumulation of antifungal activity, *E. purpurascens* was grown in Erlenmeyer flasks (250 ml) in one of the two liquid media (100 ml) on an orbital shaker (100 rev min⁻¹) at 20°C. Three replicate flasks were removed every 2 days for 20 days after inoculation. The cultures were sterilized using Nalgene filters (0.22 µm pore size, Nalge Co.) and the filtrates stored at -18°C before extraction and assaying for antifungal activity.

The culture filtrates were assayed for antifungal activity using a cup-plate method. Wells (4 mm dia) from the leading edge of colonies of test fungi were placed over the wells and the plates held at 20°C. Fungi used in this assay were *P. cinnamomi* (IMI 129910), *P. intermedium* (F) and *F. oxysporum* f.sp. *lini*. Diameters of *P. intermedium* colonies were measured 36 h after inoculation and colonies of *P. cinnamomi* and *F. oxysporum* f.sp. *lini* were measured 3 days after inoculation. Mean diameter of each colony was obtained from two measurements made at right angles. Inhibition of colony growth produced by antifungal activity was calculated as the percentage reduction in colony diameter compared with the diameter of control colonies. Reduction (%) was determined for each fungus for each sample date.

To obtain crude samples of antifungal compounds culture filtrates were acidified with conc. HCl (3 ml 1⁻¹) and extracted three times with diethyl ether, 1/3 vol of ether being used each time. The ethereal extracts were dried over anhydrous Na₂SO₄, filtered and the solvent removed under vacuum at 40°C (Raistrick and Rudman, 1956). This procedure was used to extract compounds from filtrates (200 ml) taken every 2 days for 20 days after inoculation of GAP medium and SCA medium with *E. purpurascens*. After removal of the ether, each residue was redissolved in ether (2 ml). Aliquots (100 µl) were spotted on TLC plates (Kieselgel 60F₂₅₄, Merck; 0.2 mm thick). The plates were developed in chloroform:methanol (96:4) (Baute et al., 1978). To detect antifungal activity, the plates were air dried and sprayed with a dense suspension of conidia of *Cladosporium cladosporioides* or oospores of *P. intermedium* (F) in a liquid medium containing glucose, 5 g; mycological peptone, 2 g; K₂HPO₄, 50 mg; MgSO₄·7H₂O, 50 mg in 100 ml distilled water and incubated for 2 days at 20°C. Fungitoxic compounds were located by the absence of aerial mycelium (Klarmann and Stanford, 1968).

To obtain larger quantities of the yellow phenolic antifungal compound, flavipin, (Raistrick and Rudman, 1956; Bamford et al., 1961) batches of culture filtrate (41) were extracted with diethyl ether as described. For partial purification of flavipin silica gel removed from the appropriate zone on preparative chromatograms (Chromata-lay; 100 x 200 mm; Kieselgel 60F₂₅₄, Merck, 0.5 mm) was finely ground, eluted with methanol and the methanol removed under vacuum. The residue was redissolved in known volumes of methanol or ether.

Purified flavipin was prepared from crude ether extract by sublimation at 140°C in high vacuum using a cold finger and glycerol bath (Raistrick and Rudman, 1956). A known weight of purified flavipin was dissolved in methanol and a dilution series (5 to 1 µg ml⁻¹) was prepared. A peak absorbance in u.v. radiation was recorded at 209 nm and using this wavelength a standard concentration curve was prepared. From the standard curve, concentrations of flavipin in other samples were determined following the methanol elution of preparative TLC already described.

**Spore germination assay.**

Using a standard assay method (Anon, 1943) ED₉₀ concentrations of flavipin required for the inhibition of germination of zoospores of *P. cinnamomi* (IMI 129910), oogonia of *P. intermedium* (F) and conidia...
of F. oxysporum f. sp. lini was determined. A dilution series (50 to 0.1 μg ml⁻¹) of purified flavipin (by sublimation) in ether was prepared and aliquots (20 μl) were pipetted onto clean glass slides. The ether was allowed to evaporate and onto the residues were pipetted drops of spore suspensions (20 μl, 10⁴ spores ml⁻¹) of the test fungi. Incubation proceeded for 36 h and 3 days respectively at 20°C. Two-hundred randomly-selected conidia from each drop were then counted for germination. Four drops were used for treatment and the experiment was repeated once.

Inhibition of mycelial growth of various Phytophthora spp

Toxicity of flavipin to mycelial growth of Phytophthora spp and Pythium spp was determined using the cup-plate assay already described. A dilution series of partially purified flavipin (containing 500 to 20 μg ml⁻¹ flavipin) in ether was prepared and aliquots (20 μl) were pipetted into wells (4 mm) cut in PDA (20 ml medium per plate). Six replicate plates were used for each dilution. Ether (20 μl) was similarly pipetted into wells in PDA as controls. After the ether had evaporated sterile distilled water (20 ml) was pipetted into the wells and 1 h was allowed for dissolution of the residue and partial diffusion into the agar. Discs (8 mm) cut from the leading edge of fungal colonies were placed over the wells. Incubation of Pythium spp and Phytophthora spp proceeded for 36 h and 3 days respectively at 20°C. Reduction in colony growth was determined as already described and the quantity of flavipin which caused 50% reduction in colony growth of each species was calculated.

The toxicity of filtrates of 4-day old cultures of E. purpurascens grown in SCA medium—epicorazine fraction (Baute et al., 1978)—to Phytophthora and Pythium spp was also assayed using the cup-plate method. Aliquots (50 μl) of sterilized culture filtrates (Nalgene filters) were pipetted into wells in six replicate PDA plates for each fungus. Sterile distilled water was used in control plates. Incubation proceeded as already described and reduction (%) in colony growth was calculated.

β, 1-3 glucanase and cellulase production by E. purpurascens

To determine if E. purpurascens produced β, 1-3 glucanase or cellulase using hyphal wall polysaccharides as a carbon source the fungus was grown in Erlenmeyer flasks (250 ml) containing SCA medium (100 ml) in which the sucrose was replaced by hyphal wall material from P. cinnamomi or P. intermedium. To produce hyphal wall material P. cinnamoni and P. intermedium were grown in Erlenmeyer flasks (250 ml) in SCA medium (100 ml) on an orbital shaker (100 rev min⁻¹) at 20°C for 7 days. The mycelium was removed from the cultures by filtration and homogenized in cold sterile distilled water five times to remove water soluble material. The remaining hyphal material was homogenized twice in large volumes of cold acetone and allowed to dry in a cool stream of air. The hyphal wall material (0.2 g) was included in casamino acid medium (100 ml) before autoclaving. Eighteen replicate flasks containing each hyphal wall preparation were inoculated with eight discs (4 mm) cut from the leading edge of cultures of E. purpurascens. The flasks were incubated on an orbital shaker (100 rev min⁻¹) at 20°C for 2–7 days. Three replicate flasks were taken every day, the mycelial content removed by filtration using Nalgene filters and the filtrates stored at -18°C before enzyme assay.

The substrate used for the detection of β, 1-3 glucanase contained laminarin (0.1% w/v Sigma Chemical Co.) in 0.1 M-sodium acetate buffer (pH 5). Carboxymethyl cellulose (0.1% w/v Sigma Chemical Co.) in 0.1 M-Tris/HCl buffer (pH 7) was used as substrate to assay for cellulase activity. Substrates (2 ml) were mixed with culture filtrate (2 ml) and incubated at 30°C for 3 h. Enzyme activity was determined colorimetrically with 3,5-dinitrosalicylic acid reagent (Miller, 1959).

RESULTS

Interaction between E. purpurascens and opposing fungi

From the results of direct opposition on agar between E. purpurascens and a number of fungal pathogens a considerable variation in the degree of antagonism was observed. Of four fungi reported to have been inhibited by E. purpurascens, B. allii (Bamford et al., 1961) and C. saitius (Campbell, 1956) showed mutual antagonism. Antagonism between E. purpurascens and F. oxysporum f. sp. lini was of this type. C. ulmi (Webber and Hedger, 1986) and R. solani (Chand and Logan, 1984) were inhibited but much less severely than Phytophthora spp and Pythium spp. Microscopic observation showed that overgrowth of Pythium spp by E. purpurascens resulted in considerable distortion of Pythium mycelia. Red pigmentation from the E. purpurascens colony concentrated in Pythium sp oogonia the protoplasmic contents of which had degenerated.

Four isolates of E. purpurascens were tested for their antagonistic properties on direct opposition plates and one (13) consistently inhibited opposing fungi to a greater extent than the other three isolates. Isolate 13 was used in all subsequent experiments.

Antifungal compound production by E. purpurascens

Antifungal activity was detected in filtrates from 2-day old cultures of E. purpurascens grown in GAP medium. Activity increased to a maximum in 12-day old cultures and then declined (Fig. 1). Mycelial growth of P. cinnamoni was much more sensitive to antifungal activity in this medium than that of P. intermedium and F. oxysporum f. sp. lini.

Antifungal activity was detected at Rₜ 0.16 on autobiographies of other extracts of culture filtrates from day 2 until day 16, maximum activity appearing in extracts of 10-day old culture filtrates. A second zone of inhibition appeared at Rₜ 0.61 (compound Z) on day 8, reached maximum size on day 12 and was no longer detected after day 16. The yellow compound detected at Rₜ 0.16 was believed to be the phenolic compound flavipin (Raistrick and Rudman, 1956; Bamford et al., 1961). The orange-yellow ether extract of 10-day old culture filtrates, purified by sublimation, yielded a pale-yellow compound, m.p.
The approximate concentration of flavipin produced in filtrates of cultures containing glucose and \((\text{NH}_4)_2\text{HPO}_4\) was calculated from u.v. absorbance at 209 nm of methanol eluates of the flavipin spot on preparative TLC (Fig. 2). Compound Z did not give a blue coloration with FeCl$_3$-ferricyanide reagent and no attempt was made to identify it.

The profile of antifungal activity produced by \textit{E. purpurascens} in SCA medium was very different from that in GAP medium. Maximum activity was detected in this medium only 4 days after inoculation and \textit{P. intermedium} was the most sensitive of the three test fungi (Fig. 3). However, inhibition of mycelial growth of \textit{P. intermedium} rapidly declined as the \textit{E. purpurascens} culture aged. Mycelial growth of \textit{P. cinnumomi} was slightly less inhibited by 4-day old cultures than \textit{P. intermedium} but the aging cultures continued to inhibit its growth. \textit{F. oxysporum} f.sp. \textit{lini} was again the least sensitive to antifungal activity produced in this medium.

Ether extracts of the filtrates revealed four compounds with antifungal activity in 4-day old SCA cultures (Fig. 4). Two of the zones of inhibition on the autographs, \(R_f\) 0.71 and 0.49, corresponded very closely with \(R_f\) values reported for epicorazine A and epicorazine B, respectively (Beute et al., 1978). The identities of compound X (\(R_f\) 0.90) and compound Y (\(R_f\) 0.80) are unknown and these compounds are believed, not to have been previously reported. Much less flavipin was produced in this medium than in GAP medium and was detected only in 8–12-day old cultures (Fig. 3). Compound Z, \(R_f\) 0.61, also accumulated in this medium, maximum activity being obtained from 20-day old cultures (Fig. 4).

\textbf{Inhibition of spore germination by purified flavipin}

To determine the toxicity of flavipin, purified by sublimation, to spores of the test fungi ED$_{50}$ concentrations were obtained at pH 5 (Table 1). Zoospores
of *P. cinnamonii* and oogonia of *P. intermedium* were 10–20 times more sensitive to flavipin than conidia of *F. oxysporum* f. sp. *lini* and *B. allii*. Determination of toxicity to *B. allii* was included in the assay for comparison with published data. Raistrick and Rudman (1956) obtained a value of 7.5 µg ml⁻¹ for the inhibition of 5–50% conidia of *B. allii* at pH 3.5 and 10–12.5 µg ml⁻¹ totally inhibited germination at the same pH (Raistrick and Rudman, 1956; Bamford et al., 1961).

### Toxicity of flavipin and epicorazone fraction to mycelial growth of *Phytophthora* and *Pythium* spp

From results of a cup-plate assay using dilutions of ether extract of 10-day old GAP culture filtrates in the epicorazone fraction (Table 2). Colony growth of *F. oxysporum* f. sp. *lini* was inhibited by only 14% by the epicorazone fraction and 300 µg ml⁻¹ flavipin was required to inhibit colony growth by 50% in cup-plate assays.

### Interaction between hyphae of *E. purpurascens* and *P. cinnamonii* and *P. intermedium*

As hyphae of *E. purpurascens* and *P. cinnamonii* or *P. intermedium* approached each other on gel slides, those of the two pathogenic fungi became stunted and greatly distorted as shown in Figs 5a and 6.

When hyphae of *E. purpurascens* and *P. cinnamonii* or *P. intermedium* intermingled, some hyphae of the pathogenic fungi appeared devoid of contents and therefore dead. *E. purpurascens* hyphae coiled round, penetrated and grew inside the hyphae of both fungi, although seen more strikingly with *P. cinnamonii* (Fig. 5b).

#### β-glucanase and cellulase production by *E. purpurascens*

Hyphal walls of *Phytophthora* spp and *Pythium* spp contain cellulose rather than chitin (Dietrich, 1973). The capacity of *E. purpurascens* to produce β, 1–3 glucanase or cellulase, necessary for wall penetration, was determined using *P. cinnamonii* or *P. intermedium* hyphal wall material as the sole carbon source. Isolate 13 is known to be cellulolytic, cellulase activity being readily detected in colonised plant material (Brown, 1984). Cellulase and β, 1–3 glucanase activities were detected in cultures containing the hyphal wall material.

### DISCUSSION

The isolate of *E. purpurascens* used in this study and other isolates were much more antagonistic to the concentration of flavipin required to inhibit colony growth by 50% was calculated. The concentration of flavipin in the ether extract was determined prior to the preparation of the flavipin standard curve. Mycelial growth of *Pythium* and *Fusarium* spp was inhibited to a greater extent than the *E. purpurascens* flavipin fraction (Table 2), although differences between isolates of *P. cinnamonii* and some *Pythium* spp were insignificant. Other *Phytophthora* spp tested were more tolerant of these compounds.

### Table 1. Inhibition of spore germination by purified flavipin at pH 5

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>ED₅₀ value (µg ml⁻¹)</th>
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<tbody>
<tr>
<td><em>Phytophthora cinnamonii</em></td>
<td>0.53</td>
</tr>
<tr>
<td>(IMI 129910)</td>
<td></td>
</tr>
<tr>
<td><em>Pythium intermedium</em> (F)</td>
<td>0.85</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em> Exp. lini</td>
<td>10.40</td>
</tr>
<tr>
<td><em>Bacillus allii</em></td>
<td>7.84</td>
</tr>
</tbody>
</table>

*Estimated dose required to cause inhibition of germination of 50% of spores.

#### Table 2. Inhibition of mycelial growth of *Phytophthora* and *Pythium* spp by partially purified flavipin and by epicorazone fraction

<table>
<thead>
<tr>
<th>Fungal spp</th>
<th>Flavipin conc causing 50% reduction in colony growth (µg ml⁻¹)</th>
<th>Epicorazone fraction conc reduction in colony growth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phytophthora</em> spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. cinnamonii</em></td>
<td>50.5 (5.02)*</td>
<td>57.8 (4.61)*</td>
</tr>
<tr>
<td>(IMI 129910)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. cinnamonii</em></td>
<td>40.2 (4.51)</td>
<td>52.9 (4.14)</td>
</tr>
<tr>
<td>(IMI 70473)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. cinnamonii</em></td>
<td>75.5 (5.52)</td>
<td>58.5 (4.89)</td>
</tr>
<tr>
<td>(IMI 157800)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. cactorum</em></td>
<td>30.1 (2.53)</td>
<td>32.1 (2.72)</td>
</tr>
<tr>
<td><em>P. citricola</em></td>
<td>186.3 (15.62)</td>
<td>42.8 (3.17)</td>
</tr>
<tr>
<td><em>P. cryptogaeae</em></td>
<td>125.1 (11.47)</td>
<td>27.2 (1.91)</td>
</tr>
<tr>
<td><em>P. syringae</em></td>
<td>32.3 (2.09)</td>
<td>35.6 (2.43)</td>
</tr>
<tr>
<td><em>Pythium</em> spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. intermedium</em> (F)</td>
<td>126.3 (15.46)*</td>
<td>74.1 (3.72)*</td>
</tr>
<tr>
<td><em>P. intermedium</em> (S6)</td>
<td>194.7 (22.15)</td>
<td>62.3 (3.41)</td>
</tr>
<tr>
<td><em>P. paroecae</em></td>
<td>124.2 (13.31)</td>
<td>67.2 (4.14)</td>
</tr>
<tr>
<td><em>P. irregulare</em></td>
<td>90.4 (10.36)</td>
<td>62.7 (2.74)</td>
</tr>
<tr>
<td><em>P. syphisticum</em></td>
<td>116.8 (13.87)</td>
<td>69.2 (3.83)</td>
</tr>
<tr>
<td><em>P. heteroallaiscum</em></td>
<td>102.5 (9.36)</td>
<td>61.0 (2.50)</td>
</tr>
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</table>

*20 µl flavipin solutions per well in cup-plate assay.
*Filtrate (50 µl) from 4-day old sucrose plus casamino acid cultures.
*SE in parenthesis.
Fig. 5a. Hyphae of *E. purpurascens* (Ep) approaching those of *P. cinnamomi* (Pc). Abnormal swelling of *P. cinnamomi* hyphae (hs) occurred and regions of protoplasmic degeneration (pd) were visible (×100).

Fig. 5b. Coiling of *E. purpurascens* around hyphae of *P. cinnamomi* (c) and the growth of *E. purpurascens* hyphae (Ep) inside *P. cinnamomi* hyphae (Pc) (×100).

Fig. 6. Stunted and distorted hyphae of *P. intermedium* adjacent to the boundary with an *E. purpurascens* colony (×100).
the Phycomycetes Phytophthora spp and Pythium spp, than to F. oxysporum f. sp. lini, B. allii and R. solani. Spore germination in P. cinnamomii and P. intermedium was inhibited with concentrations of flavipin 10–20 times less than that required to inhibit germination of conidia of F. oxysporum f. sp. lini and B. allii. B. allii was the test organism used in the studies of flavipin by Raistrick and Rudman (1956) and by Bamford et al. (1961). Flavipin and the epicorazine fraction were also more inhibitory to mycelial growth of Phytophthora and Pythium spp than to mycelial growth of F. oxysporum f. sp. lini. When hyphae of E. purpurascens and Pythium spp intermingled on PDA pigments produced by the epicorazine fraction were also more inhibitory to the Phycomycetes E. purpurascens. The yellow colouration of flavipin was also observed in oogonia of P. intermedium and zoospores of P. cinnamomii in spore germination assays. Such affinity between sexual structures of these fungi and pigments with antifungal activity could enhance the effectiveness of these compounds in reducing inoculum in vivo.

The epicorazine fraction, which was more toxic to Pythium spp than flavipin, comprised epicorazines A and B (Baute et al., 1978) and two other antifungal compounds (compounds X and Y), apparently hitherto unidentified. Activity attributable to the epicorazines was not detected when E. purpurascens was grown in the GAP medium or in malt broth (2%) (C. Gilmore, personal communication). Flavipin was produced in both GAP and SCA media but was preferentially produced in media, low in, or free from amino acids. A third unidentified antifungal compound (compound Z) was detected in both of the media used.

Accumulation of antifungal activity in malt broth was similar to that in GAP medium (C. Gilmore, personal communication). Royse and Ries (1978) also detected antifungal activity in cultures of E. purpurascens grown in malt extract broth. They extracted two fungitoxic compounds but apparently neither was flavipin. We did not detect the epidorins isolated from E. purpurascens cultures by Burge et al. (1976).

This considerable variation in the production of antifungal compounds in different selective media would suggest caution is necessary when attempting to equate in vitro and in vivo production of such compounds, for example, amino acids as precursors are required for the production of the diketopiperazine compounds, epicorazines A and B (Flint, 1964). The other two antifungal compounds produced in the epicorazine fraction also apparently required amino acids as precursors and may be structurally related to the epicorazines. None of the antifungal compounds detected in vitro were sought in soils inoculated with E. purpurascens. The extraction of such compounds is often difficult as many are unstable in soils, or become bound to soil particles (Jeffreys, 1952). Red pigmentation of stored preharvest retted flax stems colonised by E. purpurascens could, however, be related to production of antifungal compounds in vitro. Isolate I3 had become the dominant saprobe on these stems (Brown, 1984) outcompeting Cladosporium herbarum, Botrytis cinerea and Alternaria spp which are common colonists during the retting process (Brown and Sharma, 1984).

Coging and penetration of hyphae of both P. cinnamomii and P. intermedium was observed and extracellular B. 1,3 glucanase and cellulase activities were detected in E. purpurascens cultures containing Phytophthora or Pythium hyphal wall material as the sole carbon source. Extracellular B. 1,3 glucanase production by E. purpurascens was low compared with that produced by an isolate of the mycoparasite Trichoderma harzianum (Brown, unpublished data) but together with cellulase activity should facilitate penetration of Phytophthora and Pythium hyphal walls. Cellulolytic activity varies between isolates of E. purpurascens and Kilpatrick and Chilvers (1981) showed that two non-cellulolytic isolates were not antagonistic to their test fungus, Curvularia sp. Coging and penetration of chitin containing walls of hyphae of R. solani (Chand and Logan, 1984) and C. sativus (Campbell, 1956) have also been observed.

Disruption of the contents of Phytophthora and Pythium hyphae was observed before penetration occurred, probably resulting from antibiosis, and it is possible that E. purpurascens grew saprophytically in the dead hyphae rather than penetrating live mycelium.

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