Metabolic Changes during Development of *Phytophthora palmivora* Examined by Gas Chromatography/Mass Spectrometry

By B. R. GRANT, 1* W. GREENAWAY 2 AND F. R. WHATLEY 2

1 Russell Grimwade School of Biochemistry, University of Melbourne, Australia
2 Department of Plant Sciences, University of Oxford, Oxford, UK

(Received 9 March 1988)

Metabolic profiles from four stages of differentiation of the fungus *Phytophthora palmivora* were obtained by gas chromatography/mass spectrometry. The profiles showed the presence of sterols in the asexual reproduction stage of the organism, and confirmed their virtual absence from the mycelial stages. The zoospore stage was characterized by the presence of polyunsaturated fatty acids of C20 and C22 chain length. The transition from zoospore to cyst was also marked by the appearance of disaccharides and by a decrease in the amount of phosphate present. There were also distinctive shifts in the proportions and the total amounts of amino acids present, with γ-aminobutyrate and alanine increasing as germination took place. These distinctive profiles identify some of the metabolic changes which accompany differentiation in this fungus.

INTRODUCTION

Transmission of diseases caused by fungi of the genus *Phytophthora* generally involves the formation of motile, wall-less cells – the zoospores – which serve to disperse the fungus (Zentmyer, 1983). In order to re-establish infection the zoospore must recognize the proximity of a potential host and then respond by settling down and secreting a cell wall to form a cyst. The cyst subsequently germinates, producing a germ tube, which penetrates the host and initiates the first stage of the infection process. The characteristic changes in morphology and ultrastructure which accompany this process (Bartnicki-Garcia & Wang, 1983; Carlile, 1983; Hemmes, 1983; Paktitis et al., 1986) are paralleled by striking changes in the physiological requirements of the organism (Carlile, 1986).

The zoospore is a cell adapted to survive in a hostile environment. Its energy supply is provided solely by internal reserves, which include both lipids (Bimpong, 1975) and carbohydrates (Wang & Bartnicki-Garcia, 1980), and it is unable to utilize exogenous energy sources (Penington, 1987). Because it lacks a cell wall, the zoospore maintains its integrity against an osmotic gradient of at least 250 mosmol (Grant et al., 1986) by actively pumping water out by means of a water expulsion vacuole (Hemmes, 1983). During the transformation to the encysted state and the subsequent formation of the germling, a cell wall containing 1,3- and 1,4-β-polyglucans is secreted and the cell switches from total dependence on internal reserves to the uptake and metabolism of external sources of carbon and nitrogen (Byrt, 1980; Penington, 1987).

This transformation must be reflected in changes in the metabolism of the cell, but to date, there is little information about those changes which take place. This has been due in part to the difficulty in obtaining sufficient material for study and to the daunting task of selecting the most appropriate parts of the total metabolic sequence for detailed investigation.

The development of methods for producing large numbers of zoospores of *Phytophthora palmivora* in axenic culture (e.g. Grant et al., 1984) and for inducing controlled, synchronous

Abbreviations: TMS, trimethylsilyl; HFB, heptafluorobutyrate.
differentiation in these cells (Grant et al., 1985) has removed one of these obstacles. The technique of metabolic profiling suggests a means of dealing with the other. Metabolic profiling as originally developed utilizes the resolving power of capillary gas chromatography (GC) to separate compounds, usually as volatile derivatives, and yields a series of recognizable peaks characteristic of the steady-state concentrations of the compounds within the organism. Combined with the capacity of mass spectrometry (MS) to identify the components separated by the gas chromatograph the technique becomes very powerful (Horning & Horning, 1971). Metabolic profiling has been used to suggest the sites of action of fungicides (Whatley et al., 1977), and to indicate the reasons for many inherited inborn errors of metabolism in humans (e.g. Goodman & Markey, 1981). These latter indications were subsequently confirmed by conventional biochemical analyses. We therefore used metabolic profiling to examine the changes in various metabolites during asexual reproduction in *P. palmivora*, where a population of cells undergoes synchronous developmental succession during which individual cells become in turn zoospores, cysts and germlings.

**METHODS**

*Organism and culture.* The strain of *Phytophthora palmivora* (Butler) ATCC 26286 used here originated as a single zoospore isolate from a culture isolated from pawpaw (*Carica papaya* Tourn. ex L.). The organism was maintained at 26 ± 1 °C on V-8 agar. Mycelia were grown on cleared V-8 juice (Ribeiro, 1978) or, in a single experiment, on Miracloth pads covering Petri dishes containing V-8 agar, which allowed the mycelium to be separated from the solid medium and washed free of contaminating solubles from the medium (Byrt & Grant, 1979). Cultures were inoculated with a single cube of mycelium-agar taken from the extending edge of a 3 d old culture.

Mycelia were harvested after 7 d of growth. Those from liquid cultures were collected onto glass fibre filter pads (Whatman GF/A), washed three times with distilled water and freeze-dried overnight. The yield was typically 130 ± 10 mg per 100 ml culture medium. Mycelia produced on the Miracloth pads were grown in darkness to prevent sporulation. After 7 d growth the pads were stripped from the agar, washed three times in distilled water and homogenized in a Polytron blender. The homogenate was then washed twice more, the washings removed by centrifugation (200 g, 10 min) and the solids freeze-dried. A sample of the total freeze-dried solid (400 mg), equivalent to two plates of mycelium, was taken for extraction. Agar plugs used to seed plates or liquid cultures were removed from the mycelium at the first wash stage.

Sporangia were induced to form on 4 d old mycelium by incubation (3 d) in light. Zoospores were produced and harvested by the methods described previously (Grant et al., 1984), except that the detached sporangia were washed twice before zoospore release, and finally suspended in a solution containing: CaCl₂, 10 µM; MgCl₂, 10 µM and KCl, 100 µM. Zoospore concentrations of 2–3 × 10⁶ cells ml⁻¹ were obtained routinely by this method and a total of 1–1.5 × 10⁶ cells were required to provide the 100 mg freeze-dried material needed for each analysis.

Synchronous differentiation was induced in zoospore populations by the addition of sodium pectate (from citrus, final concentration 0.5 mg ml⁻¹) to the cell suspensions. Cysts were harvested after 20 min and germlings after a 90 min incubation at 20 °C. At this temperature differentiation was markedly slower than observed previously at 24 °C (Grant et al., 1985), but at the time of harvest more than 90% of the cell population had undergone differentiation to the appropriate stage. The progress of differentiation was monitored during the incubation using an inverted microscope.

Zoospores were harvested immediately after their release from sporangia by filtering through Whatman 541 filter paper and then snap-freezing the whole cell suspension in dry ice/acetone. Cysts and germlings were collected as a pellet by centrifugation (1000 g, 5 min), washed once in distilled water, and the concentrated suspension snap-frozen and freeze-dried. Cell material was held dry at −20 °C prior to analysis.

*Extraction, separation and derivatization.* Freeze-dried material (50 mg) was extracted and fractionated as described by Whatley et al. (1986). The procedure yielded four fractions: (1) ether soluble, (2) neutral plus cation, (3) anion and (4) zwitterion, each of which was derivatized and analysed separately. The ether fraction was saponified with hot KOH, neutralized with HCl and then freeze-dried before the formation of the trimethylsilyl (TMS) derivatives. The zwitterion fraction was divided before derivatization and one aliquot used to form the TMS ethers, while the second was used to form the more stable N(O,S) heptafluorobutyrate-isobutyl esters (HFB-isobutyl esters) of the amino acid components (Mackenzie & Tenaschuk, 1979) for quantification. The TMS ether derivatives were prepared as described by Dunstan et al. (1984).

Chromatographic separations were carried out on 30 m silica capillary columns (i.d. 0.3 mm) coated with OV-1, using a linear temperature programme from 85 °C to 310 °C at 3 °C min⁻¹, with a 10 min hold at the final temperature. The HFB-isobutyl esters of the amino acids were detected by flame ionization and identified by their retention times. Relative amounts were determined by integration of peak areas using a Hewlett-Packard 3390A reporting integrator and the relative peak areas were corrected for molar responses. The TMS ether derivatives of
the other fractions were analysed in a Finnigan 1020 automated GC/MS system, incorporating a Data General Nova 3 computer as described by Dunstan et al. (1984), the identification of the chromatographic peaks being done by comparison with a user-generated library of mass-spectra of known compounds and confirmed by the observed retention time.

RESULTS

Comparison of the metabolic profiles from the four growth stages (Figs 1, 2, 3 and 4) shows a number of distinct differences. In the saponified lipid extracts from zoospores, cysts and germings a group of compounds were present, which eluted between 3900 and 4000 s. These compounds were apparently absent from mycelial extracts run at the same concentration but could be seen to be present in a more concentrated run (Fig. 1). On the basis both of their retention times and of their mass spectra the compounds were identified as the mono-TMS derivatives of: cholest-5-en-3β-ol (cholesterol); 24-methylcholest-5-en-3β-ol (campesterol, if α-methyl; or ergosterol, if β-methyl); 24α-ethylcholest-5,22δ-dien-3β-ol (stigmasterol); and 24-ethylcholest-5-en-3β-ol (β-sitosterol, if α-ethyl; or poriferasta-5-en-β-ol, if β-ethyl). The proportions of the four sterols were in the ratio 1:2:5:5, as calculated from their relative peak areas observed with the flame ionization detector, uncorrected for specific response. They accounted for slightly less than 2% of the total lipid present. Five-fold concentration of the mycelial lipid fraction before derivatization showed the same four sterols to be present, with cholesterol in a higher proportion than in the zoospore, cyst and germling. The maximum amount of sterol in the mycelium was less than 0.1% of the total lipid present. Samples of the V-8 broth, freeze-dried and taken through the same extraction procedure showed traces of sterol. Analysis of sterol in cleared V-8 juice has previously shown a sterol concentration in the order of 4.5 µg ml⁻¹ (Nes, 1987). Samples of mycelia grown on uncleared V-8 agar on a Miracloth pad, as described in Methods, contained no more sterol than mycelia grown in liquid culture. This indicates that the reduced concentration of sterols observed in the mycelia was not due to the depletion of sterol in the medium as a result of the removal of solids when the V-8 juice was cleared.

Material eluting between 2800 and 3000 s was present in all the stages but at a much lower concentration in the mycelial stage (Fig. 3a). The component from zoospores eluting at scan 2818 is tentatively identified as eicosatetraenoic acid-mono-TMS (C_{20:4}) on the basis of its mass spectrum (Fig. 3b). Other C_{20} mono- and poly-unsaturated fatty acids were present in the zoospore fraction. In mycelia the saturated (C_{20:0}) and monounsaturated (C_{20:1}) fatty acids were the main constituents eluting in this region and polyunsaturated fatty acids were virtually absent. A further pair of compounds, eluting at around 3230 and 3275 s (not shown), were identified as TMS ethers of polyunsaturated C_{22} fatty acids. Again, these compounds were present in lower concentrations in the mycelial lipids. The C_{20} and C_{22} polyunsaturated fatty acids were present in greatest amounts in the zoospore lipid and fell to 60% of that level in the cyst and germling (Table 1). The amounts of C_{18:1} and C_{18:2} also increased markedly from mycelial to zoospore and cyst stages. The other major components of the saponified lipid fraction are listed in Table 1. The largest single component, diethylene glycol, partitioned between the lipid fraction and the neutral component of the water fraction. For convenience of presentation it is shown as being wholly within the lipid fraction. The total amount of diethylene glycol remained relatively constant in all four growth stages. The data from zoospores, cysts and germings presented in Table 1 was obtained from the analysis of a single lot of each growth stage. Three separate batches were pooled to provide each lot. To estimate the variation which might be expected between batches using this method three separate lots of mycelium were analysed, and the mean values of the components together with the SEM values shown in column 1 of the table.

The full metabolic profiles of the four neutral plus cation fractions are shown in Fig. 4. The major qualitative differences are seen in the following: in the pentose sugar TMS ether derivatives, eluting at 1500–1600 s, which were present in noticeably higher amounts in the mycelia; in the hexose sugars, almost exclusively glucose, eluting between 2000 and 2200 s,
Fig. 1. GC elution profiles of sterols extracted from different growth stages of *P. palmivora*. (b) Zoospore; (c) germling. The amounts of sterols were very low in the mycelial extract (a), which was run after fivefold concentration (see text). The peaks labelled A–E indicate those sterols that were further studied by MS (see Fig. 2).

Table 1. Changes in specific lipid components during asexual reproduction in *P. palmivora*

The values for zoospore, cyst and germling components were obtained from single, pooled samples made up of three separate batches of cells. The values for mycelial components are the mean of triplicate samples of mycelium, grown as separate batches and at different times, SEM values are given in parentheses.

<table>
<thead>
<tr>
<th>Component</th>
<th>Growth stage . . .</th>
<th>Mycelium</th>
<th>Zoospore</th>
<th>Cyst</th>
<th>Germling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylene glycol</td>
<td>17000 (4500)</td>
<td>20 300</td>
<td>21 100</td>
<td>31 000</td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>6600 (1600)</td>
<td>5 300</td>
<td>8 600</td>
<td>4 500</td>
<td></td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>9 100 (1 240)</td>
<td>11 600</td>
<td>8 700</td>
<td>7 500</td>
<td></td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1)</td>
<td>1 600 (650)</td>
<td>2 100</td>
<td>1 200</td>
<td>1 000</td>
<td></td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>14 600 (2 600)</td>
<td>20 000</td>
<td>14 100</td>
<td>12 700</td>
<td></td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>3 700 (1 400)</td>
<td>14 000</td>
<td>17 000</td>
<td>13 000</td>
<td></td>
</tr>
<tr>
<td>C$<em>{20}$–C$</em>{22}$ polyunsaturated fatty acids</td>
<td>6 000 (1 500)</td>
<td>2 300</td>
<td>1 400</td>
<td>1 400</td>
<td></td>
</tr>
<tr>
<td>Total lipid fraction</td>
<td>65 000</td>
<td>85 000</td>
<td>74 000</td>
<td>73 000</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2. Ion fragmentation patterns of the peaks A–D shown in Fig. 1. 
(a) Peak A, retention time 3954 s (cholesterol-TMS ether); (b) peak B, retention time 4093 s (campesterol-TMS ether); (c) peak C, retention time 4134 s (stigmasterol-TMS ether); (d) peak D, retention time 4209 s (sitosterol-TMS ether). Based on the ion fragmentation pattern, the unknown (peak E in Fig. 1b, c), eluting after sitosterol, is also a sterol or a sterol mixture (data not shown). The sterol pattern observed in the lipid from the cyst stage was identical to that in the zoospore and germling material.
Fig. 3. GC elution profiles for C18 and C20 polyunsaturated fatty acids extracted from mycelial lipid, where these compounds are present in low concentrations (a) and from zoospore lipid (b) from P. palmivora. (c) Ion fragmentation patterns observed from the peaks eluting at 2818 and 2905 s in (a) and (b).
Fig. 4. Total metabolic profiles of the neutral plus cation fractions from mycelia (a), zoospores (b), cysts (c) and germlings (d) from *P. palmivora*. P, TMS-pentoses; H, TMS-hexoses, principally glucose; D, TMS-disaccharides. Scylinositol and myoinositol and a group of unidentified sugar derivatives elute between H and D.
Table 2. Variation in components in the anion fraction for four growth stages of *P. palmivora*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mycelium</th>
<th>Zoospore</th>
<th>Cyst</th>
<th>Germling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>24700</td>
<td>55600*</td>
<td>45000</td>
<td>37400</td>
</tr>
<tr>
<td>C₆ Sugars†</td>
<td>38200</td>
<td>19000</td>
<td>28500</td>
<td>13000</td>
</tr>
<tr>
<td>Malic acid</td>
<td>Trace</td>
<td>—</td>
<td>3100</td>
<td>1300</td>
</tr>
<tr>
<td>Citric acid</td>
<td>Trace</td>
<td>—</td>
<td>2800</td>
<td>2200</td>
</tr>
<tr>
<td>Total anion:</td>
<td>76100</td>
<td>83500</td>
<td>93700</td>
<td>63700</td>
</tr>
</tbody>
</table>

* In this run the phosphate peak was clearly overloaded; this will have led to an underestimate of phosphate in the zoospore fraction.
† Total area of the multiple peaks representing hexose sugars.

Table 3. Relative molar concentrations of amino acids in developmental stages of *P. palmivora*

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Mycelium</th>
<th>Zoospore</th>
<th>Cyst</th>
<th>Germling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>51 (37)</td>
<td>20 (9)</td>
<td>32 (24)</td>
<td>158 (20)</td>
</tr>
<tr>
<td>Glycine</td>
<td>5 (4)</td>
<td>22 (10)</td>
<td>6 (5)</td>
<td>28 (4)</td>
</tr>
<tr>
<td>Valine</td>
<td>4 (3)</td>
<td>6 (3)</td>
<td>2 (2)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Threonine</td>
<td>7 (5)</td>
<td>11 (5)</td>
<td>3 (2)</td>
<td>16 (2)</td>
</tr>
<tr>
<td>Serine</td>
<td>9 (6)</td>
<td>18 (8)</td>
<td>6 (5)</td>
<td>20 (3)</td>
</tr>
<tr>
<td>Leucine</td>
<td>3 (2)</td>
<td>3 (1)</td>
<td>1 (1)</td>
<td>9 (1)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2 (1)</td>
<td>—</td>
<td>—</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Proline</td>
<td>17 (12)</td>
<td>39 (17)</td>
<td>14 (11)</td>
<td>138 (17)</td>
</tr>
<tr>
<td>Methionine</td>
<td>3 (2)</td>
<td>11 (5)</td>
<td>2 (2)</td>
<td>7 (1)</td>
</tr>
<tr>
<td>Aspartate</td>
<td>5 (4)</td>
<td>29 (13)</td>
<td>20 (15)</td>
<td>97 (12)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Glutamate</td>
<td>5 (4)</td>
<td>72 (31)</td>
<td>42 (32)</td>
<td>185 (23)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3 (&lt;1)</td>
</tr>
<tr>
<td>Ornithine</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4 (1)</td>
</tr>
<tr>
<td>γ-Aminobutyrate</td>
<td>28 (20)</td>
<td>—</td>
<td>4 (3)</td>
<td>100 (13)</td>
</tr>
<tr>
<td>Total:</td>
<td>139</td>
<td>231</td>
<td>132</td>
<td>790</td>
</tr>
</tbody>
</table>

* Values shown in parentheses are peak areas that have been corrected for relative molar responses of the HFB-isobutyl esters, using data given in Mackenzie & Tenaschuk (1979). These values are expressed as a percentage of the total area.

which were present in much higher amount in the cyst extract; and in the peaks eluting between 3500 and 4000 s, identified as a mixture of disaccharides and which formed major components of both cyst and mycelial fractions.

The anion fractions are compared in Table 2. Two features stand out. First, there were higher concentrations of phosphate in each of the spore stages than in the mycelial stages; the amounts decreased as the transition from zoospore to germling was completed. Secondly, there were marked increases in the concentrations of citric and malic acids (identified as the TMS derivatives) in the cyst and germling stages. These compounds were absent from the zoospore and present in only trace amounts in the mycelium. The amino acid composition, determined as the HFB-isobutyl esters from the zwitterion fraction, also showed characteristic patterns in the four stages (Table 3). The germlings contained by far the largest soluble amino acid pool, more than three times that of the zoospore, and six times that present in cyst and in mycelium. Glutamate, aspartate and proline together represented more than 50% of the amino acids present in the spore stages, while in the mycelial stage alanine and γ-aminobutyrate predominated. In contrast to these changes, the other amino acids remained in relatively constant proportions throughout.
DISCUSSION

Comparisons of the metabolic profiles from the four developmental stages of *P. palmivora* indicate several previously unreported features. The presence of sterols in the reproductive stages, in contrast with their virtual absence from the mycelial stage, was predictable since it has been clearly established that vegetative growth in *Phytophthora* sp. is independent of sterols, whereas there is a sterol requirement for both asexual and sexual reproduction (Hendrix, 1965, 1966, 1970). The GC/MS method does not permit us to distinguish the α and β-isomers of the sterols, but on the basis of the known sterol composition of this group of fungi (McCorkindale et al., 1969; Nes, 1987) we believe the four sterols present to be cholesterol, campesterol, stigmasterol and β-sitosterol. The same sterols are present in the growth medium. Sterols are reported to be generally absent from the mycelial stage of the Pythiaceae (McCorkindale et al., 1969).

Since sterols are present in the asexual reproductive stage our results raise the question of when sterol uptake begins in *P. palmivora* and how it might be regulated. The interaction between light and cholesterol supplementation observed in this species by Hendrix (1967) is consistent with the hypothesis that sterol uptake in the mycelial phase is restricted. However, this is in contrast with the results obtained with *Phytophthora cactorum* Gonzales & Parks (1981), who observed that cholesterol was accumulated by the mycelia grown in liquid culture when the sterol was offered. Differences in sterol absorption from liquid as opposed to agar cultures have been observed (Nes et al., 1983; Nes & Stafford, 1983) but did not appear to contribute to the differences noted in our results. The question is therefore whether sterol uptake in *P. palmivora* is accelerated during sporangiogenesis, or whether there is a redistribution into the developing sporangium of the very limited amount of sterol accumulated during the vegetative phase.

The presence of polyunsaturated fatty acids of C₂₀ and C₂₂ chain lengths in the spore stage has not apparently been observed previously although a high proportion of the fatty acids present in the mycelial lipids of *Saprolegnia parasitica* were of this type (Gellerman & Schlenk, 1979) and they were also isolated from the mycelium of *Phytophthora infestans* (Bostock et al., 1981), where they acted as elicitors to phytoalexin production in the potato. Although the shorter chain length fatty acids are present in much larger amounts and their degree of unsaturation increases on spore formation, it is the apparent absence of the C₂₀ and C₂₂ polyunsaturated fatty acids from the mycelium, their presence in the zoospore and their rapid decrease over the 20 min period when zoospores develop into cysts which marks them as worthy of special attention and suggests that they are compounds which have a specific function in the zoospore stage.

The changes in the sugar concentrations, and specifically their increase in the cyst stage in comparison with zoospore and germling stages, can be interpreted in terms of what has been proposed to account for the rapid cell wall formation at this stage. Cyst formation is characterized by the formation of a polyglucan cell wall from endogenous precursors (Bartnicki-Garcia & Wang, 1983). The glucose units are derived from phosphomycaminarin, the major reserve polysaccharide in the zoospore stage (Wang & Bartnicki-Garcia, 1980). The size of this molecule would exclude it from the profiles determined by our methods. However, the rapid increase in both monosaccharides and disaccharides observed in the cyst stage is consistent with Bartnicki-Garcia & Wang's proposal that zoospore mycolaminarin is dephosphorylated and degraded into small units prior to assembly into the cyst wall, rather than being used as a block polymer within the wall assemblage. The activation of a phosphatase and a 1,3-β-glucan hydrolase must therefore be an essential feature of the transformation of zoospore to cyst. A future study of these two enzymes could reveal useful markers of this transition.

The large amount of phosphate characteristic of the spore stages may originate, in part, from the phosphomycaminarin and partly from unstable sugar phosphates, which latter degrade during the ion-exchange fractionation step. However, the presence of the very large amounts of phosphate suggests an appreciable concentration of the free anion or its unstable precursors in the spore stages. Subsequent to this work, ³¹P-NMR spectra of perchloric acid extracts of mycelia have been shown to be particularly rich in polyphosphates (J. O. Niere personal communication).

The presence of both malic and citric acids in cyst and germling stages is consistent with a
glyoxylate cycle being switched on at these stages, providing a means of converting endogenous lipid to sugar at a period when rapid cell wall biosynthesis is required but exogenous sugars are not yet available. Such a conclusion, based solely on pool sizes of malate and citrate, will need confirmation by future estimates of the fluxes involved, as well as estimates of the key enzymes of the glyoxylate cycle, isocitrate lyase and malate synthetase. These two enzymes could be useful markers for the late cyst to early germling stage of development.

The changes in amino acid concentrations also suggest alterations in metabolism characteristic of each stage. There is a fall in total free amino acid concentration in the transition from zoospore to cyst, a transition that is known to be cycloheximide insensitive (Hemmes & Hohl, 1971; Penington, 1987). Thus the decrease probably reflects a burst of protein synthesis immediately after encystment. The massive increase in the free amino acid concentration in the germling stage suggests that proteolysis has become extremely active, degrading the spore proteins and providing amino acids for synthesis of the new proteins characteristic of the mycelial stage. Investigation of these proteases and the study of their regulation is clearly an important prerequisite to understanding the latter stages of the transition from the free-living to the parasitic phase of Phytophthora. In addition to these changes in total amino acid pool size, the increase in the proportion of alanine and the appearance of γ-aminobutyrate as the spore develops into mycelium suggest changes in the fluxes through specific pathways of amino acid metabolism. The change in concentration of γ-aminobutyrate, a breakdown product of arginine, suggests a switch in the pattern of arginine metabolism as differentiation proceeds.

We believe that a study of metabolite profiles at the various stages of development of Phytophthora may provide important clues as to the major metabolic pathways operating. Only those compounds which separated as TMS derivatives on OV-1, and which differed in concentration at the different stages of development, were detected in these experiments. More labile intermediates need to be isolated by other extraction techniques, e.g. perchloric acid extraction, when this is indicated. Metabolic profiling can only make use of changes that are seen. Fruitful clues for further biochemical studies may thus be revealed, but there can be no guarantee that every significant change will be revealed by this approach. The enormous amount of data in each profile, of which only the most obvious have been presented here, remains available for continuous re-evaluation, as more detailed investigations take place.

The authors gratefully acknowledge the contribution of Tracy Scaysbrook in the preparation of material for analysis, and of Professor W. R. Nes to the discussion of sterol distribution. The strain of P. palmivora used in this work was imported into the UK with the permission of the Ministry of Agriculture Fisheries and Food and used under licence no. PHF/124 (76).

REFERENCES


specificity in accumulation of sterols by Phytophthora cactorum. Lipids 16, 384–388.


