Comparative Studies of the Mineral Nutrition of Three Species of Phytophthora

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SUMMARY

Nutritional experiments were carried out dealing with: (1) the utilization of sulphur compounds; (2) the utilization of phosphorus compounds; (3) the action of different mineral salts and their interaction. The results showed that Phytophthora erythroseptica grew well at 28° and pH 6·6 incubated for 20 days; P. parasitica grew well at 28° and pH 6·6 for 17 days; and P. infestans at 20° and pH 4·5 for 16 days. In the adjusted controlled medium pH changes were generally within one pH unit. The best carbon and nitrogen sources are stated. The only satisfactory sulphur sources for P. infestans were sodium sulphate and sodium thiosulphate. These compounds were also among the best S sources for P. erythroseptica and P. parasitica, but a number of other compounds also were utilized equally well, e.g. sodium metabisulphite, sodium sulphite, α-cysteine, sodium sulphide, sodium dithionite and methionine. For all organisms the best phosphorus sources were sodium dihydrogen orthophosphate, sodium metaphosphate and lecithin. Rate of utilization of the phosphorus was an important factor in mycelial yield. Factorial experiments were carried out in which P. erythroseptica and P. parasitica were incubated at 28° for 17 days. Statistical analysis of the results showed that under the given conditions optimal growth measured as mg. dry wt. was obtained in liquid media containing glucose, 25 g./l.; DL-asparagine, 4·0 g./l.; FeSO₄.7H₂O, 0·001 g./l.; thiamine, 0·8 mg./l.; ZnSO₄.7H₂O, 1 p.p.m.; H₂MoO₄, CuSO₄.5H₂O, 0·02 p.p.m.; with varying amounts of K₂HPO₄, MgSO₄.7H₂O and CaSO₄.2H₂O for the different species specified. There was a balance between all combinations of K₂HPO₄, MgSO₄.7H₂O and CaSO₄.2H₂O for P. parasitica but not for P. erythroseptica, but there were significant interactions between the salts taken two at a time for both these fungi. There was interaction for all three salts together for both species. Phytophthora infestans and P. parasitica are more exacting in their nutritional requirements than P. erythroseptica.

INTRODUCTION

Previous work at Newcastle-upon-Tyne (see Fothergill & Raine, 1954; Fothergill & Ashcroft, 1955; Fothergill & Yeoman, 1957; Fothergill & Jones, 1958; Fothergill & Hide, 1962) indicated that for some parasitic fungi the balance of the major inorganic salts in a culture medium was more important for the good growth of the mycelium than the concentration of individual salts, but that with some
saprophytic species the balance was much less important. For a wide range of fungi, however, the results varied. The object of the present experiments was to continue these investigations, with three species of the genus Phytophthora which are usually regarded as facultative parasites having only a short saprophytic existence in the soil. While a large amount of work has been done on these species, particularly P. infestans concerning taxonomy, cytology and host-parasite relationships, little comparative work seems to have been done on their mineral nutrition. Earlier experiments, such as those of Payette & Perrault (1944), were of small value from the nutritional aspect because of the use of undefined media. More exact investigations of these fungi were carried out by Hall (1959), Sakai (1955, 1956) and Mehrotra (1950).

METHODS

The following organisms were used: Phytophthora infestans (Mont.) de Bary, P. erythroseptica Pethybridge and P. parasitica Dastur. All of them were obtained from the Central Bureau voor Schimmelcultures, Baarn. Phytophthora infestans is the cause of Late Blight disease of potato; P. erythroseptica is a cause of Pink Rot of potatoes, 'shanking' in forced tulips, wilt in Atropa belladonna and other diseases; P. parasitica attacks Rieinus communis and causes 'damping off' and 'Foot Rot' of tomato and other plants. Throughout this paper the following abbreviations are used: P. infestans is designated PI; P. erythroseptica, PE, and P. parasitica, PP.

Initially single spore isolates of each of these species were prepared according to the method of Hall (1959) and stock cultures were maintained in 1 oz. screw-topped bottles each containing 10 ml. French bean oatmeal agar. The stock cultures were stored at 5° and subcultured at 3-monthly intervals, or when required. No alteration of morphological and cultural characteristics of these fungi occurred during the experimental period. In all experiments cultures were grown in 20 ml. medium in 150 ml. Erlenmeyer flasks. To avoid caramelization of media, sterilization was carried out at 115° for 10 min. After the growth period, the mycelia were filtered on to tared Whatman No. 5 filter-papers and washed with water. The mycelium with the filter paper was then dried overnight at 80–85°, cooled in a desiccator and weighed. Results are expressed as mg. dry wt. mycelium/flask, average of 4 or 5 replicates. It was not practicable to separate the mycelium from the filter paper after filtration, and, as the filter papers used lost 5% of their original weight on drying under the above conditions, appropriate allowances were made. For preliminary experiments the followed basal defined liquid medium A was used. This was a modification of a medium successfully used by Lopatecki & Newton (1956) for organisms PE and PP, in which the amounts of glucose and thiamine present were varied as follows: glucose, 25 g./l.; DL-asparagine, 4·0 g./l.; K2HPO4, 1·0 g./l.; MgSO4.7H2O, 0·1 g./l.; CaSO4.2H2O, 0·1 g./l.; FeSO4.7H2O, 0·001 g./l.; thiamine, 0·8 mg./l.; ZnSO4.7H2O, 1 p.p.m.; H2MoO4, CuSO4.5H2O and MnSO4.4H2O at 2 p.p.m. This solution was initially at pH 4·7.

Experiments were made to determine the best method of inoculation. It was found that a minced mycelium technique gave the best and most consistent results, giving a low coefficient of variation between replicate cultures and affording some control over the amount of fungal material added to each experimental flask. In this method, cultures were blended for 10 sec. with 50 ml. sterile distilled water in a
Nutrition of Phytophthora

Monel metal container of an ‘Atomix’ blender. The resulting homogenate was then centrifuged for 5 min. at 3000 rev./min. in tubes with Oxoid metal caps; the supernatant liquid was discarded and the mycelium was taken up in sterile distilled water. This washing process was repeated three times. The mycelium was finally added to a known volume of sterile distilled water, 10 ml. removed and added to an EEL colorimeter tube and the reading recorded. By reference to a previously calibrated graph with a linear scale, the quantity of blended mycelium per ml. could be calculated and the volume of the remainder of the mycelial suspension adjusted to give a concentration of 1 mg. of blended mycelium/ml. water. One ml. of suspension obtained in this way was used as the inoculum in all subsequent experiments.

Preliminary experiments indicated that the optimum temperature for organisms PE and PP was 28°, and 20° for PI. These temperatures were used in subsequent experiments. PE grew faster at all temperatures than either PP or PI which had the slowest growth rate. With medium A maximum dry wt. mycelium was obtained with PE after 20 days of incubation, with PP after 13 days, and after 16 days with PI. These incubation times were used in subsequent experiments unless otherwise stated.

Further experiments with medium A showed that glucose, asparagine, KH₂PO₄ and MgSO₄.7H₂O were essential nutrients for all three fungi. The omission of CaSO₄.2H₂O from the medium decreased the growth of PE by 70%, of PI by 50% and of PP by only 7%. The omission of the trace elements had little effect on growth-yield of PP but seriously affected that of the other two species. Omission of FeSO₄.7H₂O had a very marked effect on the yield of PI and a considerable effect on PP and PE.

The growth of PE and PP in this medium resulted in an increase of acidity as great as 3.5 pH units. The optimum pH for both PP and PE was 6-6, that for PI was 5-0. Medium A was thus subsequently modified by omission of the initial concentration of KH₂PO₄ replacing it with Sorensen’s salt adjusted to give pH 6-6 (i.e. with KH₂PO₄, 4.746 g./l. + Na₂HPO₄.2H₂O, 5.443 g./l.). This medium is referred to as medium B and was used subsequently for organisms PE and PP. Sorensen’s salt at any concentration had a distinctly depressing effect on the growth of PI and medium A at pH 4-7 was used for this fungus.

Tests showed that none of the fungi needed an exogenous supply of biotin, pyridoxine, nicotinamide, folic acid, riboflavin, calcium pantothenate, p-aminobenzoic acid, inositol or ascorbic acid; but thiamine was necessary for good growth. These results confirm those of earlier workers (Robbins, 1937; Cantino, 1955). Preliminary experiments with a wide range of structurally different carbon compounds also showed that the best carbon sources for these fungi were those of similar constitution, namely, sucrose, glucose and mannose for organisms PP and PI, and sucrose, glucose and fructose for PE. Similarly the best nitrogen sources in order of mycelial yield were as follows: for PE glycine, calcium nitrate, cystine, ethyl aminoacetate hydrochloride, histidine, asparagine; for PP aspartic acid, glycine, calcium nitrate, asparagine, γ-aminobutyric acid, ethyl aminoacetate hydrochloride; and for PI γ-aminobutyric acid, citrulline, glutamine, arginine and asparagine.
RESULTS

Sulphur requirements

While Mehrotra (1950) made a survey of the sulphur requirements of some species of Phytophthora, the results were criticized by Cantino (1955) because of a lack of pH control during the growth period. The sulphur requirement of the Phytophthora species used in the present work was thus investigated by varying the sulphur sources individually in media A and B. The sulphur compounds were each added in turn to these media to give a final concentration of 0.03 g./l. The basal media were modified by the replacement of $\text{MgSO}_4\cdot7\text{H}_2\text{O}$, $\text{CaSO}_4\cdot2\text{H}_2\text{O}$, $\text{FeSO}_4\cdot7\text{H}_2\text{O}$ and trace elements containing sulphur with $\text{MgCl}_2\cdot6\text{H}_2\text{O}$, $\text{CaCl}_2\cdot6\text{H}_2\text{O}$ and $\text{FeCl}_3\cdot6\text{H}_2\text{O}$ to give final concentrations of 0.1, 0.1 and 0.001 g./l., respectively. Preliminary experiments showed that the addition of chloride ion as KCl did not affect the growth of these fungi. The modified medium A was inoculated with organism PI and the medium B with organisms PE and PP as previously described. Organisms PE and PP were incubated at 28° and PI at 20°. Dry weights of 5 replicate cultures were determined after 9, 13 and 17 days of incubation for PP, after 15, 20 and 25 days for PE, and after 16 days for PI. The final pH values of

Table 1. The utilization of inorganic and organic sulphur compounds by Phytophthora species

Highest yields expressed as percentages of yields from $\text{Na}_2\text{SO}_4\cdot10\text{H}_2\text{O}$ taken as 100. Initial pH value for Phytophthora erythroseptica (PE) and P. parasitica (PP) = 6.6; for P. infestans (PI) = 4.7. Final pH values recorded in table. (Mg. dry wt. mycelium/flask average of 5 replicates. Incubation period for PI 16 days; those for PE and PP shown in brackets.)

<table>
<thead>
<tr>
<th>Species</th>
<th>Organism PE (mg. dry wt./flask and incubation time)</th>
<th>Final pH value</th>
<th>Organism PP (mg. dry wt./flask and incubation time)</th>
<th>Final pH value</th>
<th>Organism PI (mg. dry wt./flask at 16 days)</th>
<th>Final pH value</th>
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<td>100 (17) 5.7</td>
<td>100 (25) 5.8</td>
<td>100 (17) 5.7</td>
<td>100 (25) 5.8</td>
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<td>75 (25) 5.5</td>
<td>100 (17) 5.5</td>
<td>75 (25) 5.5</td>
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<td>110 (17) 5.5</td>
<td>124 (25) 5.5</td>
<td>110 (17) 5.5</td>
<td>124 (25) 5.5</td>
<td>124 (25) 5.5</td>
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<td>90 (17) 5.3</td>
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<td>Cystine</td>
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<td>127 (25) 5.7</td>
<td>108 (17) 5.4</td>
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<td>Sulphanilic acid</td>
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<td>15 (18) 5.6</td>
<td>20 (20) 6.2</td>
<td>15 (18) 5.6</td>
<td>20 (20) 6.2</td>
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<td>Sulphamic acid</td>
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<td>14 (18) 5.4</td>
<td>16 (20) 5.8</td>
<td>14 (18) 5.4</td>
<td>16 (20) 5.8</td>
<td>16 (20) 5.8</td>
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<tr>
<td>Sulphanilamide</td>
<td>10 (25) 6.0</td>
<td>16 (18) 5.5</td>
<td>10 (25) 6.0</td>
<td>16 (18) 5.5</td>
<td>10 (25) 6.0</td>
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<td>22 (9) 5.5</td>
<td>18 (25) 6.1</td>
<td>22 (9) 5.5</td>
<td>18 (25) 6.1</td>
<td>18 (25) 6.1</td>
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<td>Methionine</td>
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<td>104 (17) 5.4</td>
<td>110 (25) 5.8</td>
<td>104 (17) 5.4</td>
<td>110 (25) 5.8</td>
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</tr>
<tr>
<td>Thiazamide</td>
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<td>17 (17) 6.0</td>
<td>20 (20) 6.2</td>
<td>17 (17) 6.0</td>
<td>20 (20) 6.2</td>
<td>20 (20) 6.2</td>
</tr>
<tr>
<td>Control (no sulphur)</td>
<td>17 (20) 6.1</td>
<td>16 (17) 5.5</td>
<td>17 (20) 6.1</td>
<td>16 (17) 5.5</td>
<td>17 (20) 6.1</td>
<td>17 (20) 6.1</td>
</tr>
</tbody>
</table>
the media were also determined. For organisms PE and PI there was a general increase in acidity varying between 0.4 and 1.5 pH units, while for PI the increase in acidity was never greater than 0.8 pH unit, but an increase in alkalinity as high as 2.8 pH units was sometimes recorded. The results are shown in Table 1 which records the highest percentage yields expressed in terms of the yield from Na₂SO₄. 10H₂O as 100%. With Na₂SO₄.10H₂O the actual highest mycelial yields were 155 mg. after 17 days of incubation for organism PP, 94 mg. after 20 days for PE, and 21 mg after 16 days for PI.

Since persulphate breaks down to sulphate very easily in solution, the results for potassium persulphate must be treated with reserve. In general, the results indicated that sodium sulphate and sodium thiosulphate were very good sulphur sources for all three fungi, while sodium metabisulphite, sodium dithionite, sodium sulphite, sodium sulphide, cystine and methionine were good sources for organisms PE and PP, but gave only moderate or poor mycelial yields with organism PI. Essentially similar observations were made by Fothergill & Hide (1962) with four species of Pythium. Thus, in general, the oxidation level of the sulphur atom had no consistent significant effect on mycelial growth; a thiosulphate (S₂O₃²⁻), a metabisulphite (S₂O₅²⁻), a sulphite (SO₃²⁻) and a sulphate (SO₄²⁻) were all good sulphur sources. The yields from the sulphur-containing methionine and cystine were as high or nearly so, as the yields from the best inorganic sulphur sources in contrast to the findings of Volkonsky (1933) who worked with aquatic Saprolegniales. The other compounds used, particularly those containing a benzene ring (i.e. sulphanilic acid, thiazamide, sulphanilamide, sulphosalicylic acid), were very poor sulphur sources for all three fungi, except that moderate yields of mycelium were obtained with organism PI. These results are similar to those obtained by Steinberg (1941) with *Aspergillus niger*. No further correlation of structure with utilization could be made with the fungi used, thus emphasizing their ability to utilize a wide range of sulphur-containing compounds. Preliminary experiments indicated that the metabolism of organisms PE and PP, as expressed by their nutritional requirements, was very similar and differed considerably from that of organism PI. The results with the sulphur compounds have emphasized this distinction. Organism PI shows a much narrower range of sulphur utilization than organisms PE and PP, but it seems to possess an ability to use compounds which are not used to any great extent by the other two fungi.

### The requirement for phosphorus

The importance of phosphorus in the growth of fungi is, of course, well known, but few comparative studies on the use of different phosphates by species of Phytophthora have appeared in the literature. Hence an experiment was done to study the effects of different phosphates on mycelial production. In this experiment medium A was used in which potassium dihydrogen orthophosphate was replaced by K₂SO₄. 0.67 g./l. To this medium were added singly in turn various phosphorus compounds to give a final concentration of phosphorus 0.3 g./l. The medium was otherwise unbuffered at initial pH 4.7 and the final pH value, recorded in each case, showed an increase in alkalinity of 1.0-1.5 pH units. As before, dry weights of 5 replicate cultures were determined after 9, 18 and 17 days of incubation for organism PP, after 15, 20 and 25 days for PE, and after 16 days for PI. The results are shown in Table 2 which records the highest mycelial yields expressed as a percentage
of that for sodium dihydrogen orthophosphate taken as 100%. With NaH₂PO₄ the actual highest mycelial yields were 148 mg. for organism PE after 25 days of incubation, 178 mg. for PP after 17 days, and 45 mg. for PI after 16 days.

For all organisms sodium dihydrogen orthophosphate, sodium metaphosphate and lecithin were excellent sources of phosphorus, while tetra-sodium pyrophosphate, sodium β-glycerophosphate and nucleic acid gave high yields with organisms PE and PP, but the corresponding yields for organism PI were greatly diminished. The highest comparative yields were obtained with lecithin for organism PE, and nucleic acid for organism PP but, as a whole, the inorganic phosphorus sources seemed to be better than the organic compounds. The remaining compounds gave poor yields of mycelium. There were not great differences in yield with favourable compounds but differences were noted in the rate of utilization of the compounds. For example, the two orthophosphates and sodium β-glycerophosphate were utilized much more rapidly than sodium metaphosphate or tetra-sodium pyrophosphate. Similar results have been recorded for species of Pythium by Hide (1961) and for Rhizopus stolonifer by Yeoman (1954). This suggests that orthophosphate is more readily available to these fungi and that metaphosphate and pyrophosphate are first converted in the mycelium to orthophosphate before they are utilized. Casein was a good source of phosphorus and gave visibly good mycelial growth but measurement was difficult because of heavy precipitation in the medium after incubation for 1 or 2 days; this precipitate was insoluble in water, n-HCl or H₂SO₄ and dry weights were not recorded.

**Table 2. The utilization of various phosphorus compounds by Phytophthora species**

Highest yields expressed as percentages of yields from NaH₂PO₄ taken as 100. Initially all pH 4.7. (Mg. dry wt. mycelium/flask average of 5 replicates.)

<table>
<thead>
<tr>
<th>Organism</th>
<th>P. erythroseptica (mg. dry wt./flask and incubation time, days)</th>
<th>P. parasitica (mg. dry wt./flask and incubation time, days)</th>
<th>P. infestans (mg. dry wt./flask incubation time, days)</th>
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</thead>
<tbody>
<tr>
<td>Sodium dihydrogen orthophosphate</td>
<td>100 (25)</td>
<td>100 (17)</td>
<td>100</td>
</tr>
<tr>
<td>Sodium metaphosphate</td>
<td>94 (25)</td>
<td>96 (17)</td>
<td>129</td>
</tr>
<tr>
<td>Tetra-sodium pyrophosphate</td>
<td>101 (25)</td>
<td>97 (18)</td>
<td>4</td>
</tr>
<tr>
<td>Sodium β-glycerophosphate</td>
<td>100 (20)</td>
<td>87 (18)</td>
<td>7</td>
</tr>
<tr>
<td>Sodium phosphite</td>
<td>8 (20)</td>
<td>19 (17)</td>
<td>4</td>
</tr>
<tr>
<td>Sodium hypophosphite</td>
<td>11 (20)</td>
<td>14 (18)</td>
<td>13</td>
</tr>
<tr>
<td>Nucleic acid</td>
<td>97 (20)</td>
<td>116 (17)</td>
<td>49</td>
</tr>
<tr>
<td>Lecithin</td>
<td>119 (20)</td>
<td>77 (18)</td>
<td>81</td>
</tr>
<tr>
<td>Casein</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Control (no phosphate)</td>
<td>23 (20)</td>
<td>11 (19)</td>
<td>11</td>
</tr>
</tbody>
</table>

+ , Good mycelial growth but weight not determinable.

**Factorial experiments**

Some of the basal physical and chemical requirements of a culture medium for the growth of the organisms PE and PP having been determined, the effect of the balance of the chemicals used in the medium was next investigated. The factorial design and statistical analysis of the results should indicate whether or not a balance
Nutrition of Phytophthora

between the salts is necessary for high mycelial yields under the given experimental conditions. The direct effect of the individual salts in the medium and the interaction between them is also determined. Previous experiments showed that medium A was a satisfactory medium for organisms PE and PP but KH₂PO₄ was replaced by K₂HPO₄ at a concentration of 1.046 g./l. to give initial solutions nearer to the pH growth optimum for these fungi. The solution was adjusted to pH 6.6.

Initial factorial experiments showed that the highest concentrations of the salts were possibly limiting growth and this medium A was further modified to give basal concentrations of K₂HPO₄ at 1.568 g./l., of MgSO₄·7H₂O at 0.41 g./l. and of CaSO₄·2H₂O at 0.414 g./l. The new solutions were also adjusted to pH 6.6. In all experiments the final pH values of all replicates were determined; the variation of pH value was never more than one pH unit. The cultures were incubated at 28° for 17 days for organisms PE and PP. The concentrations of the salts were fixed on the basis of halving and doubling those in the basal medium containing K₂HPO₄. Thus K₂HPO₄, MgSO₄·7H₂O and CaSO₄·2H₂O were each used at three concentrations and all possible combinations of them were set up, giving a total of 27 variations. Each combination was done in quintuplicate, thus, with each species of fungus, 135 culture flasks in each experiment were incubated. Each 150 ml. Erlenmeyer flask contained 30 ml. medium. The results given are the average mg. dry wt./mycelium of 5 replicates. The grouped results are shown in Table 3 and the grouped analyses of variance are given in Table 4. For abbreviations of the salt concentrations used in the text below see Table 3.

Table 3. Growth responses to two species of Phytophthora to different conditions of K₂HPO₄, MgSO₄·7H₂O and CaSO₄·2H₂O

<table>
<thead>
<tr>
<th>K₂HPO₄ and MgSO₄·7H₂O conditions</th>
<th>P₁</th>
<th>P₂</th>
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<tr>
<td>Mg₁                 Mg₂                 Mg₃</td>
<td>PE</td>
<td>PP</td>
<td>PE</td>
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<td>Mg₁                 Mg₂                 Mg₃</td>
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<td>Mg₁                 Mg₂                 Mg₃</td>
<td>PE</td>
<td>PP</td>
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</table>

Mean dry wt. mycelium/flask

From the single salt analysis it is evident that there was no consistent result with all salts for both organisms. This analysis showed that the differences between mycelial dry weight with K₂HPO₄ were significant at all concentrations of the salt for both organisms, and in both cases the highest yield was obtained at the P₁ level of the salt. For MgSO₄·7H₂O with both organisms the differences observed between the Mg₁ and Mg₂ levels of this salt were significant. For organism PE the highest mean weight was obtained at the Mg₁ level, while for organism PP the Mg₂ level
P. G. FOTHERGILL AND J. H. CHILD
gave the highest yield. For CaSO₄·2H₂O with organism PE there were significant
differences between the mean mycelial weights at the Ca₁ and Ca₂ level and also at
the Ca₂ and Ca₃ levels, but not between the Ca₁ and Ca₃ levels of this salt. For
organism PE these latter levels gave the same mycelial yield but for organism PP
the highest mean yield was obtained with the Ca₃ concentration.

Table 4. Analysis of variance (grouped) for Phytophthora erythroseptica (PE) and
P. parasitica (PP)

| Required 'F' and 't' values taken from Snedecor's tables (1946). Sums of squares and mean squares are omitted from table. |
|---|---|---|
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| | | |

The grouped analyses of variance showed that there were significant interactions
between all the salts in the first order combinations for both organisms. These
results show that there was a physiological balance in some cases between these
salts under the given conditions. With organism PE the interaction between
K₂HPO₄ and MgSO₄·7H₂O showed that at the P₁ and P₂ levels increasing the
concentration of MgSO₄·7H₂O had only a small effect. The highest yield was obtained
with the highest concentration of K₂HPO₄ and the lowest concentration of MgSO₄·
7H₂O; no balance is indicated. But with organism PP similar dry weights were
obtained at the P₁ and P₃ levels with the Mg₃ level and, in general, increasing the
concentration of K₂HPO₄ led to increasing yields relative to an increasing level of
MgSO₄·7H₂O; a balance between the salts is indicated. The interaction of MgSO₄·
7H₂O and CaSO₄·2H₂O is significant but there were only relatively small gross
differences between the yields of PE. The highest yields were obtained with the
Mg₁ and Ca₃ levels and with Mg₃ and Ca₁ levels. The Ca₂ levels did not show any
consistent change in yield with increasing concentration of MgSO₄·7H₂O. With
organism PP the interaction of these two salts is interesting. At the Ca₁ and Ca₂
levels, increasing the MgSO₄·7H₂O concentration had little effect on yield and there
were no significant differences between them, but with the Ca₃ level there appeared
to be inhibition of growth with the lowest concentration of MgSO₄·7H₂O. Doubling
the amount of this salt in the medium gave a great increase in mycelial yield
which was maintained, but not improved, by further increase in concentration of
MgSO₄·7H₂O. A balance between these two salts is necessary and seems to be
operative in the ratio of 2 CaSO₄·2H₂O:1 MgSO₄·7H₂O. With organism PE the
interaction between K₂HPO₄ and CaSO₄·2H₂O showed that at the P₁, P₂ and P₃
levels there was increasing yield with increasing concentration of CaSO₄·2H₂O, but
at the P₁ level the highest yield was given with the Ca₃ level, while at the P₃ level it was given with the Ca₁ level. With organism PP at the Ca₃ level there was no consistent growth increase with increasing K₂HPO₄ concentration and the highest yield was obtained at the P₃ and Ca₉ levels. The yields were nearly identical at the P₁Ca₉, P₃Ca₉ and P₅Ca₉ levels. Thus a balance is indicated.

The analysis of variance also showed that the interaction of all three salts was significant for both organisms. The concentration of K₂HPO₄ was here the most important factor controlling the yield of the organism PE. The highest yields were obtained at the P₃ level. At the P₁ level increasing the concentration of the other two salts only affected the yield slightly. At the P₃ and Ca₉ levels high yields were only obtained when the MgSO₄·7H₂O concentration was low. With organism PP there was little difference between the mean mycelial yields at the P₅Mg₁Ca₉ and P₅Mg₅Ca₉ levels. There was a consistently increasing yield as the concentration of the salts was increased proportionately from the P₁Mg₂Ca₁, P₃Mg₂Ca₂ and the P₅Mg₅Ca₉ levels. The highest mean weight of 191 mg. was obtained at the P₅Mg₂Ca₉ level, but weights of 186, 187 and 181 mg. were also obtained at the P₅Mg₅Ca₁, P₅Mg₅Ca₉ and P₅Mg₅Ca₉ levels, respectively, suggesting that the concentration is not so important when the other two salts are in a relatively high concentration in the approximate ratio of 2CaSO₄·2H₂O:1MgSO₄·7H₂O.

**Balance between MgSO₄·7H₂O and CaSO₄·2H₂O**

The previous experiments indicated that there was a physiological balance between K₂HPO₄ and CaSO₄·2H₂O. While balance has been shown to exist between various combinations of salts by different workers, this would seem to be the first time a balance has been indicated between these two salts. To confirm this result another experiment was designed to test the change in the amount of growth which might result when the concentrations of the salts were varied while still maintaining the balance between them. This experiment was designed factorially following the method of Talley & Blank (1941) and Fothergill & Ashcroft (1955). Thus the basic concentrations of MgSO₄·7H₂O and CaSO₄·2H₂O were 0·205 and 0·414 g./l., respectively; these were decreased to half in one set of solutions and increased twice and then four times in other solutions. This gave four treatments each having the same balance between the salts but the ratio of their concentrations was 0·5:1:2:4. Each of these four solutions was tested singly with K₂HPO₄ at 2·091, 3·136, 4·181 and 5·227 g./l., respectively, giving 16 solutions in all. The remaining ingredients of the basal medium remained the same. The medium was adjusted initially to pH 6·6 and the cultures incubated in 4-replicate at 28° for 11 days. The results expressed as mg. mean dry wt. mycelium per flask are shown in Table 5 where treatments are numbered 1–16 in parentheses.

The results showed that the mean mycelial yields for K₂HPO₄ at the P₁, P₂, P₃ and P₄ levels were not significantly different from each other but they were different from the yields at the Mg₁ and Ca₁ levels. To show the interaction of MgSO₄·7H₂O and CaSO₄·2H₂O 't' tests were performed. There were no significant differences between treatments 2, 3 and 4 at the P₁ level, but there was between treatments 1 and 2. At all other concentrations of K₂HPO₄ a similar pattern was shown, that is, there were no significant differences between treatments 6, 7 and 8, treatments 10, 11 and 12, and treatments 14, 15 and 16; but at the P₅, P₆ and P₇ levels, respec-
In general, there were significant differences between treatments 14, 15 and 16. At the
P2, P3 and P4 levels, respectively, there were significant differences between treat-
ments 5 and 6, 9 and 10, 13 and 14 at these levels of K2HPO4. These results confirm
the conclusion that a balance between MgSO4·7H2O and CaSO4·2H2O exists. But
since significantly different mean dry weights were recorded with the Mg1 and Ca1
levels at all levels of K2HPO4, it is evident that the effect of the balance between
MgSO4·7H2O and CaSO4·2H2O operated only after a certain concentration of the
salts had been reached. Thereafter the concentration of either of these salts could
be varied without affecting the mycelial yield of Phytophthora parasitica, provided
that the concentration of the other salt is varied proportionately.

Table 5. Mean dry weights and growth responses of Phytophthora parasitica after
11 days incubation at 28° on media with the same balance but with different concentra-
tions of certain components

Concentrations of K2HPO4: P1, P2, P3, P4 = 2·091, 3·136, 4·181, 5·227 g./l.; of
MgSO4·7H2O : Mg1, Mg2, Mg3, Mg4 = 0·102, 0·205, 0·410, 1·620 g./l.; of CaSO4·
2H2O: Ca1, Ca2, Ca3, Ca4 = 0·202, 0·414, 0·828, 1·656 g./l. ‘t’ = 2·447 for 6 de-
grees of freedom at P = 0·05; values of ‘t’ for combinations of treatments, 1 × 2 =
4·068, 2 × 3 = 0·390, 3 × 4 = 0·917, 5 × 6 = 7·170, 6 × 7 = 0·780, 7 × 8 = 1·764,
9 × 10 = 4·176, 10 × 11 = 0·258, 11 × 12 = 0·249, 13 × 14 = 17·159, 14 × 15 = 0·599,
15 × 16 = 0·532. Figures in brackets are treatment numbers, i.e. 1–16, referred to in
text.

<table>
<thead>
<tr>
<th></th>
<th>Mg1</th>
<th>Mg2</th>
<th>Mg3</th>
<th>Mg4</th>
<th>Mean N</th>
</tr>
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<tbody>
<tr>
<td>Mg1</td>
<td>114 (1)</td>
<td>152 (2)</td>
<td>154 (3)</td>
<td>140 (4)</td>
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</tr>
<tr>
<td>Mg2</td>
<td>97 (5)</td>
<td>173 (6)</td>
<td>169 (7)</td>
<td>148 (8)</td>
<td>148·7</td>
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<tr>
<td>Mg3</td>
<td>106 (9)</td>
<td>141 (10)</td>
<td>144 (11)</td>
<td>146 (12)</td>
<td>134·2</td>
</tr>
<tr>
<td>Mg4</td>
<td>92 (13)</td>
<td>166 (14)</td>
<td>157 (15)</td>
<td>147 (16)</td>
<td>140·5</td>
</tr>
</tbody>
</table>

General mean = 139·6 mg. dry wt. mycelium/flask.

CONCLUSION

In general, the results of the factorial experiments showed that there was a
considerable difference between the nutritional requirements of Phytophthora
erythroseptica (PE) and P. parasitica (PP). With the individual salts used in the
medium the greatest effect on mycelial growth was produced by varying the
concentration of K2HPO4 for both organisms. Results for the other two salts were
more variable and significant differences in dry weights did not vary in any general
way. An absolute balance between the major mineral nutrients studied was not
shown for P. erythroseptica but was shown for all combinations of salts with P.
parasitica. The latter fungus seems to have more specific nutritional requirements
than the former. The interaction of CaSO4·2H2O and MgSO4·7H2O at higher
concentrations was outstanding for P. parasitica. Although the omission of CaSO4·
2H2O from the original medium A had only a small effect on growth, the increase
in acidity of the unbuffered medium during growth was great. Hence the interac-
tions of CaSO4·2H2O and MgSO4·7H2O may only occur at, or near to, the optimum
growth pH for P. parasitica. Second-order interactions between three salts were
demonstrated.
The results for *Phytophthora erythroseptica* and *P. parasitica* support the findings of other workers. For example, Talley & Blank (1941), Fothergill & Ashcroft (1955) and Fothergill & Hide (1962) found that a physiological balance between mineral salts in the medium was necessary for good growth of the fungal parasites *Phymatotrichum omnivorum* and *Venturia inaequalis*, and the two species *Pythium debaryanum* and *P. ultimum*. No balance, however, was required for *P. afrertile* and *P. torulosum*. The highest mycelial yields (237 mg. for organism *P. erythroseptica* and 191 mg. for *P. parasitica*) were obtained with the modified liquid basal medium A containing glucose, 25 g./l.; DL-asparagine, 4·0 g./l.; FeSO₄·7H₂O, 0·001 g./l.; thiamine, 0·8 mg./l.; ZnSO₄·7H₂O, 1 p.p.m.; H₂MoO₄ and CuSO₄·5H₂O, 2 p.p.m. with K₂HPO₄, 0·018 g./l.; MgSO₄·7H₂O, 0·0008 g./l.; and CaSO₄·2H₂O, 0·0048 g./l. for organism PE and K₂HPO₄, 0·018 g./l.; MgSO₄·7H₂O, 0·0016 g./l., and CaSO₄·2H₂O, 0·0024 g./l. for *P. parasitica*. *Phytophthora infestans* has the most exacting nutritional requirements but grows satisfactorily on fewer sulphur and phosphorus compounds than either *P. parasitica* or *P. erythroseptica*. Mycelial yields of *P. infestans* were also very much less than those for the other fungi for all variations tried of the two basal media A and B. The highest mycelial yield for *P. infestans* was only 58 mg. and was obtained with medium A above but containing K₂SO₄, 0·67 g./l. and NaPO₃, 0·3 g./l. in place of K₂HPO₄.

REFERENCES


